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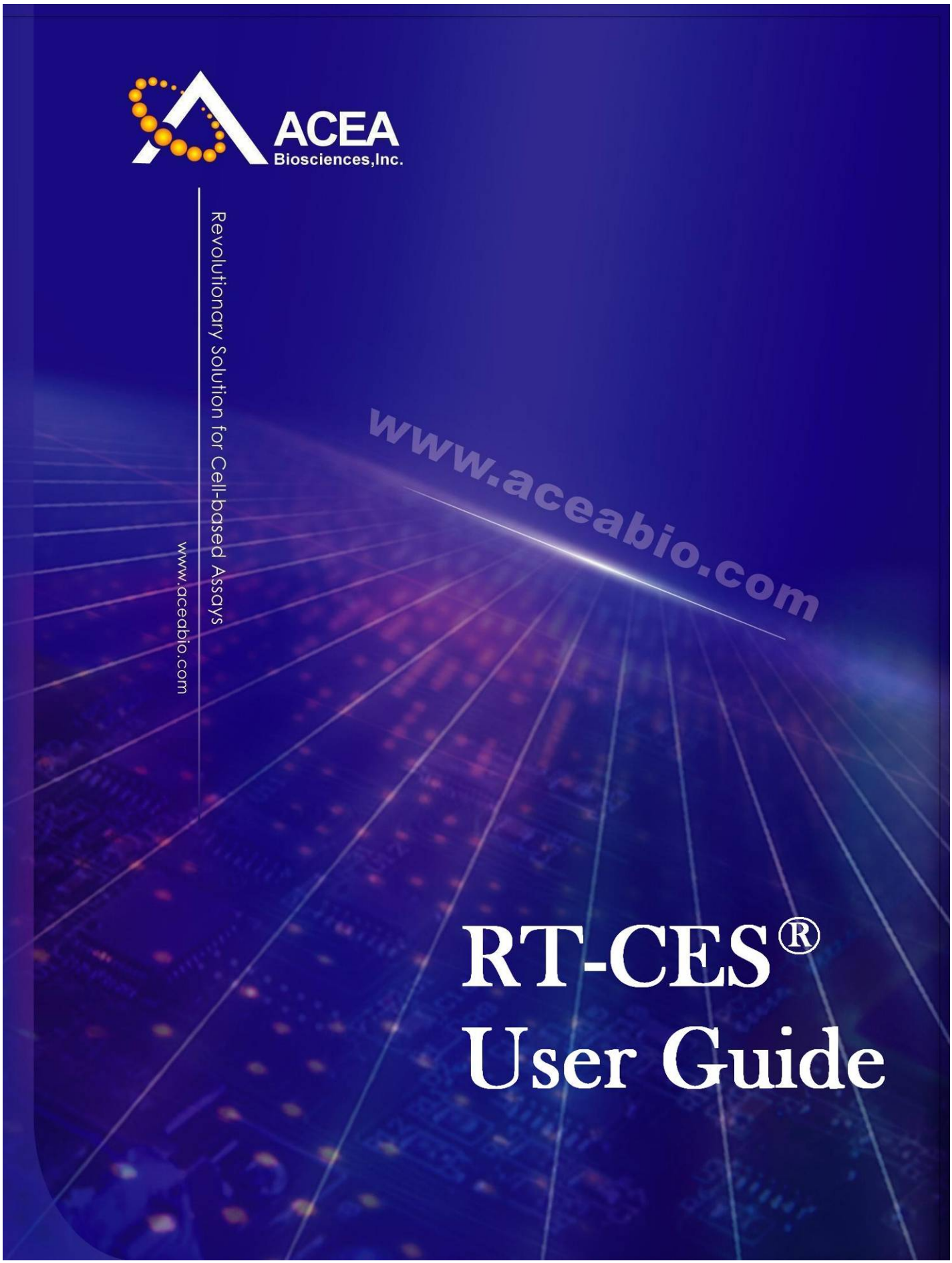
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RT-CES® System

Real-Time Cellular Electronic Sensing

User Guide

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Preface

Thank you for using ACEA Biosciences' RT-CES® system, a novel microelectronic biosensor system for label-free, real-time, automated monitoring of cell status in a variety of cell-based assays.

This User's Guide explains the RT-CES® System. Please read this User's Guide in its entirety prior to operating the system.

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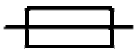
This User's Guide was prepared for users having entry-level knowledge of Windows® based programs.

Note: This User's Guide describes how to perform tasks in Windows 2000/NT/XP®. Refer to your Windows® manuals for screens and tasks specific to your version of the Windows® Operating System.

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Symbols

The following symbols may be found on the ACEA Biosciences RT-CES and RT-CIM Systems. Their definitions are as follows:

Symbol	English	Français	Deutsch
~	Alternating Current	Courant alternatif	Wechselstrom
	Power ON Power OFF	Mise sous tension Mise hors tension	Netz EIN Netz AUS
	Protective Earth Ground	Mise à la terre	Schutzerdung
	Caution: Refer to included safety statements	Attention : consulter les déclarations de sécurité incluses	Achtung: Beigefügte Sicherheitshinweise beachten
	Fuse: Refer to included safety statements	Fusible : consulter les déclarations de sécurité incluses	Sicherung: Beigefügte Sicherheitshinweise beachten
	**Do not place equipment in waste bin**. Contact ACEA for proper disposal.	**Ne pas mettre le matériel à la poubelle.** S'adresser à ACEA pour les modalités de mise au rebut.	**Gerät nicht im Hausmüll entsorgen.** Wegen ordnungsgemäßer Entsorgung ACEA kontaktieren.
COM	Communications Port	Port de communications	Kommunikations-anschluss
EPS	E-Plate® Station Port	Port de station E-Plate®	E-Plate®-Stationsanschluss
TP1/TP2	Analyzer Test Point Connections	Connexions du point de test de l'analyseur	Messpunktanschlüsse des Analysegeräts
EPS1/EPS2	E-Plate Station Connection	Connexion de station E-Plate	E-Plate-Stationsanschluss

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Regulatory Compliance

This equipment has been tested and found compliant with the following regulatory standards:

EN 61326	Conducted and Radiated Emissions
EN 61000-3-2	Power Line Harmonics
EN 61000-3-3	Power Line Flicker
IEC 61000-4-2	Electrostatic Discharge Immunity
IEC 61000-4-3	Radio Frequency Immunity
IEC 61000-4-4	Electrical Fast Transient Burst Immunity
IEC 61000-4-5	Power Line Surge Immunity
IEC 61000-4-6	RF Common Mode Immunity
IEC 61000-4-11	Voltage Dips and Short Interruptions Immunity
FCC, CFR 47, Section 15.107 Class “A”	Conducted Emissions
FCC, CFR 47, Section 15.109 Class “A”	Radiated Emissions
CAN/CSA-C22.2 No. 61010-1	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements
UL 61010-1	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements
ISA S82.02.01	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements
IEC 61010-1	Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use; Part 1: General Requirements

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General Warnings

Inspect the packaging and equipment prior to installation. Any damage to the equipment may affect its performance and should be reported to ACEA Biosciences immediately.

This equipment must be operated by trained personnel only. There are no user-serviceable components inside the equipment. Any service and/or repairs should be performed by trained ACEA personnel only. User servicing of the equipment may result in the potential risk of personal injury and void the manufacturer's warranty (the first year warranty or additional year warranty under a service contract).

	The RT-CES® Analyzer (the Analyzer) and AC adaptors for the computer and for MultE-Plate station are to be connected only to a grounded AC supply using the supplied chord as described in the Installation section of this manual. Failure to observe the described installation instruction may result in the potential risk of electrical shock and/or fire.

The RT-CES System is intended for use only in a controlled laboratory environment, as described in the Specifications section of this manual. Use of this equipment in any manner other than expressly described in this manual may adversely affect results, safety, and/or regulatory compliance.

The Analyzer must be installed in such a way to allow access to the rear panel power switch.

Do not place anything within 4 inches (100mm) of the side ventilation ports of the Analyzer. Failure to observe this distance may result in overheating of the equipment and the potential risk of fire.

	The RT-CES Analyzer is equipped with 2-pole fusing. Prior to servicing fuses, ensure that the unit's power switch is in the off "O" position and disconnect the incoming AC power chord. Replace fuses only with the type and rating shown on the Power/Fuse label on the rear panel of the instrument and in the Maintenance and Troubleshooting section of this user's manual. Failure to do so may result in the potential risk of fire and/or personal injury.

	EU Directive 2002/96/EC places restrictions on the disposal of certain electronic equipment. Do not dispose of ACEA equipment in waste receptacles. Contact your ACEA representative for proper disposal and recycling. The ACEA corporate office may be contacted directly at 866-308-2232.

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Avertissements généraux

Inspecter l'emballage et le matériel avant l'installation. Tout dommage du matériel peut nuire à ses performances et doit être immédiatement signalé à ACEA Biosciences.

Ce matériel ne doit être manipulé que par du personnel formé à cet effet. Ce matériel ne contient aucun composant réparable par l'utilisateur. Tout entretien et/ou réparation doit être ne doit être effectué que par du personnel ACE formé à cet effet. L'entretien de ce matériel par l'utilisateur peut être à l'origine d'un risque de blessure et annuler la garantie du fabricant (la garantie de la première année ou la garantie de l'année supplémentaire dans le cadre du contrat d'entretien).

	L'analyseur RT-CES® (l'analyseur) et les adaptateurs CA de l'ordinateur et de la station MultE-Plate ne doivent être branchés que sur une source d'alimentation en CA mise à la terre en utilisant le cordon fourni comme l'indique la section Installation de ce manuel. Faute de respecter les consignes d'installation, il peut exister un risque de choc électrique et/ou d'incendie.

Le système RT-CES ne doit être utilisé que dans l'environnement contrôlé d'un laboratoire, comme l'indique la section Caractéristiques techniques de ce manuel. L'utilisation de ce matériel de toute autre manière que celle qui est explicitement décrite dans ce manuel peut nuire aux résultats, à la sécurité et/ou à la conformité avec les réglementations.

L'analyseur doit être installé de manière à laisser libre accès au commutateur de tension du panneau arrière.

Ne rien placer à moins de 10 cm des évents latéraux de l'analyseur. Faute de respecter cette distance, le matériel peut surchauffer et il existe un risque d'incendie.

	L'analyseur RT-CES est équipé de fusibles bipolaires. Avant de remplacer les fusibles, vérifier que le commutateur de tension de l'appareil est en position « O » et débrancher le cordon d'alimentation CA entrant. Ne remplacer les fusibles que par des fusibles du type et de la capacité indiqués sur l'étiquette Power/Fuse du panneau arrière de l'appareil et dans la section Entretien et dépannage de ce manuel d'utilisation. Faute de quoi, il peut exister un risque d'incendie et/ou de blessure.

	La directive EU 2002/96/EC limite la mise au rebut de certains matériels électroniques. Ne mettez pas ce matériel ACEA au rebut dans les conteneurs à ordures. Adressez-vous au représentant d'ACEA pour une mise au rebut et un recyclage corrects. Il est possible d'appeler directement le siège social d'ACEA au +1 866-308-2232.

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Allgemeine Warnhinweise

Verpackung und Gerät vor der Installation überprüfen. Schäden am Gerät können die Leistung beeinflussen und sollten ACEA Biosciences umgehend gemeldet werden.

Dieses Gerät darf nur von geschulten Mitarbeitern bedient werden. Im Gerät befinden sich keine vom Benutzer wartbaren Teile. Service- und Reparaturarbeiten dürfen nur von geschulten ACEA-Mitarbeitern durchgeführt werden. Servicearbeiten am Gerät durch den Benutzer können zu Verletzungen führen und machen die Herstellergewährleistung nichtig (die Gewährleistung während des ersten Jahres oder die Gewährleistung für zusätzliche Jahre unter einem Servicevertrag).

	Das RT-CES®-Analysegerät (das Analysegerät) und die Netzadapter für den Computer und für die MultE-Plate-Station dürfen nur an eine geerdete Netzstromversorgung mithilfe des mitgelieferten Kabels wie im Abschnitt "Installation" dieses Handbuchs beschrieben angeschlossen werden. Nichtbeachtung der beschriebenen Installationsanleitung kann zu Stromschlag und/oder Brandgefahr führen.

Das RT-CES-System ist nur für den Gebrauch in einer kontrollierten Laborumgebung gedacht, wie im Abschnitt "Spezifikationen" dieses Handbuchs beschrieben. Die Verwendung dieses Geräts in einer anderen Art und Weise als in diesem Handbuch ausdrücklich beschrieben kann die Ergebnisse, Sicherheit und/oder Einhaltung gesetzlicher Vorschriften negativ beeinflussen.

Das Analysegerät muss so installiert werden, dass der Zugang zum Netzschalter an der Rückseite gewährleistet ist.

Es dürfen keine Gegenstände näher als 100 mm von den seitlichen Lüftungsöffnungen des Analysegeräts entfernt aufgestellt werden. Nichtbeachtung des Abstands kann zu Überhitzung des Geräts und Feuergefahr führen.

	Das RT-CES-Analysegerät ist mit einer 2-phasigen Sicherung ausgestattet. Vor Austausch der Sicherungen darauf achten, dass sich der Netzschalter des Geräts in der Position "O" befindet und das Netzkabel vom Strom trennen. Sicherungen nur durch Sicherungen des Typs und der Nennleistung austauschen, die auf dem Netz-/Sicherungsschild an der Rückseite des Instruments und im Abschnitt "Wartung und Fehlerbehebung" in diesem Benutzerhandbuch angegeben sind. Nichtbeachten kann zu Brandgefahr und Verletzungen führen.

	Die EU-Richtlinie 2002/96/EC enthält Einschränkungen zur Entsorgung bestimmter elektronischer Geräte. ACEA-Geräte nicht im Hausmüll entsorgen. Wenden Sie sich wegen ordnungsgemäßer Entsorgung und Recycling an Ihren ACEA-Vertreter. Sie erreichen die ACEA-Unternehmensniederlassung direkt unter 866-308-2232.

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System Description

General Overview

ACEA Biosciences has developed proprietary micro-electronic sensor technologies for monitoring cells and biomolecules. Over 95% of current biological assays are based on optical detection (e.g., fluorescent and optical detection in fluorescent plate reader, optical microscopy, FACS analyzer, fluorescent scanner). ACEA technology features the integration of molecular and cell biology with microelectronics, and is based on the electronic detection of biological assay processes.

The presence, absence, or change in properties of cells or molecules affects the electronic and ionic passage on sensor surfaces. Measuring the electronic properties of sensor surfaces provides important information about the biological status of cells (or proteins) present on the sensors. When there are changes to the biological status of the cells or proteins, analogue electronic readout signals are measured automatically in real time, and are then converted to digital signals for processing and analysis. The major advantages of the ACEA platform technology for biological assay detection over conventional approaches include:

- Label-free system
- Non-invasive, real-time monitoring
- Fully automated assay process
- High information content
- High sensitivity and accuracy

The microelectronic sensing system developed at ACEA represents one of the first integrations of electronic methodology and cell-based assays on a unique platform. This technology looks to become a benchmark item for cell biology and molecular biology labs, and is forming a new industrial standard for life science lab-ware.

RT-CES®

Real Time Cell Electronic Sensing (RT-CES®) System is a proprietary microelectronic biosensor system for cell-based assays. The core of the system is the microelectronic cell sensor arrays that are integrated in the bottom of microtiter plates (E-Plate®). The electronic impedance of sensor electrodes is measured to allow monitoring and detection of changes in cells on the electrodes. Electronic impedance of an electrode is primarily determined by the ion environment, both at the electrode/solution interface, and in the bulk solution. Under the electrical field application, the ions undergo field-directed movement and concentration-gradient driven diffusion. The total electrode impedance has two components, the resistance of the electrolyte solution (the contribution of the solution reactance is small and thus is ignored), and the impedance at the electrode/solution interface. The presence of the cells affects the local ionic environment at the electrode/solution interface, leading to an increase in the electrode impedance. In this case, the total electrode impedance has three components: the resistance of the electrolyte solution, the impedance of the cell, and the impedance at the electrode/solution and the electrode/cell interfaces. Thus, the more the cells there are on the electrodes, the larger the change in electrode impedance.

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Furthermore, the impedance change also depends on the extent to which cells attach to the electrodes. For example, cell spreading resulting in a large cell/electrode contact area leads to a large change in impedance. The cell's biological status, including cell viability, cell number, cell morphology, and adhesion degree affect the measurement of electrode impedance.

The RT-CES System consists of:

- RT-CES analyzer
- E-Plate stations (16x or 96x) or MultE-Plate Station
- Integrated RT-CES SP software
- E-Plates (16x or 96x)

Cells are introduced into the wells of the E-Plates, making contact and attaching to the sensor-electrode surfaces. The electronic properties of the sensor surfaces are monitored through an E-Plate station, which can be placed inside a tissue culture incubator, and an RT-CES electronic analyzer. This is done under the control of the integrated RT-CES SP software, to provide real time, quantitative information about the biological status of the cells, including cell number, viability, morphology and cytoskeletal dynamics.

The RT-CES system is applicable for a wide variety of cell-based assay applications, including:

- Cancer biology
- Cell adhesion and spreading
- Receptor-ligand binding and signal transduction analysis
- Cell proliferation
- Cytotoxicity
- Cellular differentiation
- Analysis of microbial and environmental toxins

The System is highly quantitative, with excellent accuracy, precision, and ease of use. The integrated software allows simplified experimental design and set up under full instrument control, with fully automated data acquisition, data analysis, and data presentation.

RT-CIM™

The RT-CIM™ (Real Time Cell Invasion/Migration) System is an extension and further development of RT-CES System for cell invasion and migration assays. The core of the system is the RT-CIM device, composed of an upper chamber (or a top chamber) and a lower chamber (or a bottom chamber). The upper chamber has 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate (PC) or polyester (PET) membrane. The membrane contains microelectronic sensor arrays that are integrated on the bottom surface of the membrane. The lower chamber serves as a reservoir for media. Cells are added to the upper chamber and are being detected by the microelectrodes on the membrane after they migrate/invade through pores on the membranes. Similar to the RT-CES system, detection of cell invasion and migration on RT-CIM system does not require labels or reporters. There is no washing, no staining and no fixation steps. Cells are continuously and automatically monitored and measured - no tedious cell counting is needed.

The RT-CIM System consists of:

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- RT-CES analyzer (the same analyzer as that used in RT-CES system)
- RT-CIM station
- Integrated RT-CES SP software (the same software as that used in RT-CES system)
- RT-CIM devices, upper chamber and lower chamber

The RT-CIM system is applicable for a range of cell migration and invasion assays. It is highly quantitative, with excellent accuracy, precision, and ease of use. It utilizes the same software (RT-CES SP) as that in RT-CES system, and allows simplified experimental design and set up under full instrument control, with fully automated data acquisition, data analysis, and data presentation.

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Component Description

RT-CES® Analyzer

RT-CES analyzer is an electronic analyzer that can, under the control of RT-CES software, conduct electronic impedance measurement of sensor electrodes. The analyzer is capable of computer-controlled signal generation, processing and analysis, automatic frequency scanning and rapid measurement – 60 and 10 sec. for 96 well plates, respectively with a W200 and W300 RT-CES analyzer.



E-Plate® Stations

E-Plate stations are placed inside the incubator and used to connect E-Plates to the RT-CES analyzer. Appropriate connection and precision electronic switches in the E-Plate station enable software control or selection of individual electrode sensors within an E-Plate to be measured by the analyzer.

For the 16x E-Plate station, there are 6 individual, zero-insertion-force (ZIF) sockets for six 16x E-Plates. Above each socket, a green light indicator signals when a 16x E-Plate is inserted properly into the socket.



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For the 96x E-Plate station, there are 120 individual, spring-loaded POGO pins, each for connecting to one of the 120 conductive pads on the back of a 96x E-Plate.



For the MultE-Plate station, there are six independent modules, each of which consists of a motor-controlled mechanism and can accommodate one 96x E-Plate. Using RT-CES MP software, one, two or more up to six users can use a W300 analyzer with MultE-Plate station for running single or multiple plate experiment.



RT-CIM™ Station

RT-CIM stations are placed inside the incubator and used to connect RT-CIM devices (including top chamber and bottom chamber) to the RT-CES analyzer. Appropriate POGO-pin connections and precision electronic switches in RT-CIM stations enable software control or selection of individual microelectrodes on RT-CIM devices to be measured by the analyzer.

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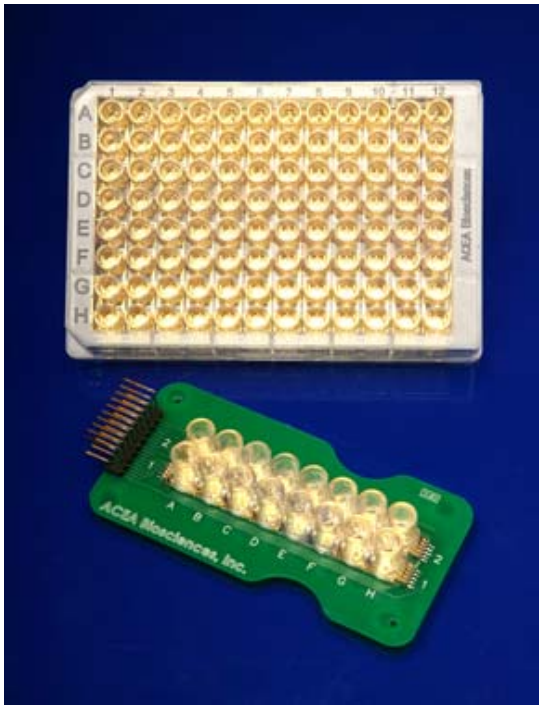
For the 16x RT-CIM station, there are 6 individual modules for six 16x RT-CIM devices. For each module, there are 20 individual, spring-loaded POGO pins, each for connecting to one of the 20 conductive pads on a RT-CIM device.



E-Plates

E-Plates are one-time use, disposable devices used for performing cell-based assays on the RT-CES system. Individual wells on the E-Plates have incorporated sensor electrode arrays so that cells inside each well can be monitored and assayed.

For 16x E-Plates, the 16 wells are arranged in an 8-row by 2-column layout, with well-to-well spacing of 9 mm. The 96x E-Plates have the same footprint and layout as conventional microtiter plates.



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RT-CIM Devices

RT-CIM devices are one-time use, disposable devices used for performing cell invasion or cell migration assays on the RT-CIM™ system. The device comprises an upper chamber (or, a top chamber) and a lower chamber (or, a bottom chamber). The upper chamber consists of 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate (PC) or polyester (PET) membrane. Microelectronic sensor arrays are integrated on the bottom surface of the membrane. The lower chamber serves as a reservoir for media. Cells are added to the upper chamber and are being detected by the microelectrodes on the membrane after they migrate/invade through pores on the membranes.



RT-CES SP and MP Software

The RT-CES SP software provides unparalleled instrument control for flexible experiment setups, data acquisition, and data analysis. All instrument and experiment controls are embedded in the software to simplify instrument setup and operation. RT-CES SP software is used with RT-CES analyzer in conjunction with 16x E-Plate station, 96x E-Plate station (single 96x plate) or 16x RT-CIM station.

The RT-CES® SP software consists of seven function pages:

- Experiment Notes
- Layout
- Test Time
- Cell Index
- Plot
- Data Analysis
- Message

The Experiment Notes, Layout, and Test Time pages contain functions required for running experiments. The Cell Index, Plot, Data Analysis and Message pages contain functions for

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monitoring and analyzing data during and after the experiments. The software provides the complete solution for dynamic measurement of cell status in the wells. Based on specific assay requirements, the measurements can be performed in any time interval, from less than one minute to days or weeks. The real-time data acquisition, data normalization, and data-plotting functions enable continuous monitoring of an experiment through the entire course of an assay. Comprehensive kinetic monitoring is a standard integrated software feature that makes the RT-CES® SP system fully automated and easy to use.

The RT-CES MP software is used with RT-CES analyzer in conjunction with MultE-plate station. The MP software was developed based on RT-CES SP software with additional functions to facilitate the measurement of up to six E-Plates. Main changes are:

A motor control and plate status page is added for controlling the status of six motor-modules and for displaying the measurement status for each plate;

For each E-plate, a 96-graph page is added for displaying the cell index curves for each individual well;

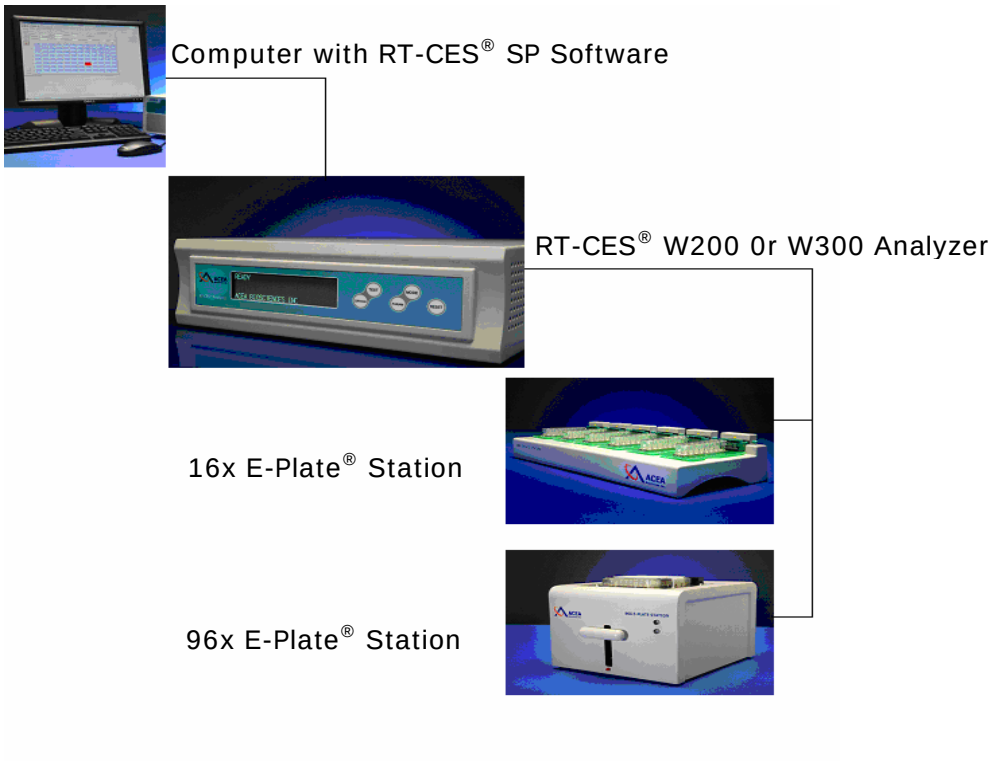
For each E-plate, there are six functional pages (the same pages as those in SP software, but without the Data Analysis page).

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System Connection

Note: Connect ONLY to a grounded power source (3-prong).

RT-CES SP System



RT-CES MP System



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RT-CIM System



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Specifications

Note: All technical specifications are subject to change without notice.

RT-CES Analyzer

- Dimension: 400 mm W x 402 mm D x 137 mm H
- Weight: 7.8 kg
- Electrical Input: 100 – 240 VAC, 50-60 Hz, 65 W max
- Output test signal: 22 mV rms ± (2% + 5 mV rms) @ 10, 25 and 50 kHz
- Impedance Measurement Accuracy: ± (2% + 1.5ohm)
- Impedance Measurement Repeatability: 0.5% for W200 and 1.5% for W300
- Impedance Dynamic Range: 20 ohm to 10 kohm
- Communication: RS232 Serial communications at a baud rate of 9600 (W200) and 57600 (W300) bits/second
- Environment: 5˚C to 40˚C, Relative Humidity: 80% max up to 31˚C decreasing linearly to 50% max at 40˚C

16x E-Plate Station

- Dimension: 375 mm W x 160 mm D x 70 mm H
- Weight: 1.8 kg
- Electrical Input: + 5V, - 5V, 5 W max
- Electronic Switch Resistance: 2 – 5 ohm
- Electrical Interface: handling six 16x E-Plate devices
- Communication: RS232 Serial communications at a baud rate of 9,600 or 57,600 bits/second
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

96x E-Plate Station

- Dimension: 228 mm W x 255mm D x 180 mm H
- Weight: 2.7 kg
- Electrical Input: + 5V, - 5V, 5 W max
- Electronic Switch Resistance: 2 – 5 ohm
- Electrical Interface: handling one 96x E-Plate device
- Communication: RS232 Serial communications at a baud rate of 9600 or 57,600 bits/second
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

16x E-Plate Station

- Dimension: 395 mm W x 200 mm D x 115 mm H
- Weight: 3.8 kg
- Electrical Input: + 5V, - 5V, 5 W max

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- Electronic Switch Resistance: 2 – 5 ohm
- Electrical Interface: handling six 16x RT-CIM devices
- Communication: RS232 Serial communications at a baud rate of 9,600 or 57,600 bits/second
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

MultE-Plate Station

- Dimension: 438 mm W x 438 mm D x 254 mm H
- Weight: 16 kg
- Electrical Input: + 12 V, DC, 12 W max (only 1 module required to move at a given time)
- Electrical Interface: handling six 96x E-Plate® devices
- Mechanism Cycle Life: 1,000 MCBF (mean cycle before failure)
- Plate interface time: less than 5 seconds for each plate
- Keypad: capable of moving the Plate up or down, indicating the plate status, indicating the motor circuit power and error state
- Communication: RS232 Serial communications at a baud rate of 57,600 bits/second with W300 Analyzer and at a baud rate of 9,600 bits/second with a COM port on computer for Motor control
- Measurement Rate: <10 seconds per measurement for one 96x E-Plate or < 1 minute per measurement for six 96x E-Plates
- Electronic Switch Resistance: 2 – 5 ohm
- Environment: 15 ˚C to 40˚C, Relative Humidity: 98% maximum, non-condensing

RT-CES SP Software Package

- Experiment Note Page: records key information about the experiment
- Experiment Layout Page: well description, chemical compound screening protocol setup
- Test Time Setting Page: measurement time interval scheduling, multiple experimental steps management
- Cell Index Page: continuous data acquisition and display
- Experimental Data Plot Page: real-time data analysis, plotting and organization of experimental data
- Data Analysis Page: Multiple calculation functions such as EC50/IC50, cell index doubling time, cell index slope
- Message Page: display error or warning messages and events during the experiment

RT-CES MP Software Package

- Motor Control and Plate Status Page: control of six motor-modules, display the measurement status for each plate
- Well-Graph Page: Selectable from Motor Control and Plate Status Page, 96-graphs each of which showing a cell index curve for one of 96-wells.

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- For each plate, there are six functional pages (the same as RT-CES SP software):
    - Experiment Note Page: records key information about the experiment
    - Experiment Layout Page: well description, chemical compound screening protocol setup
    - Test Time Setting Page: measurement time interval scheduling, multiple experimental steps management
    - Cell Index Page: continuous data acquisition and display
    - Experimental Data Plot Page: real-time data analysis, plotting and organization of experimental data
    - Message Page: display error or warning messages and events during the experiment

16x E-Plate Device

- Dimension: 107.5 mm L x 51 mm W x 21 mm H (with plate cover)
- Spacing: The spacing of the wells is to be 9mm center-to-center per the ANSI/SBS 4-2004 standard for 96-well titer plates
- Volume: 335µl ± 5µl
- Bottom Diameter: 5.0mm ± 0.05mm
- Electrical Interface: interface with 16x E-Plate device station
- Sensor Impedance: 17ohm ± 5ohm @ 10kHz, when measured with a 1X PBS Solution
- Material: biocompatible surfaces
- Sterile
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

96x E-Plate Device

- Footprint: complying with ANSI/SBS 1-2004 requirements.
- Dimension: 127.7 mm L x 85.5 mm W x 17.5 mm H (with plate cover)
- Spacing: The spacing of the wells is to be 9mm center-to-center per the ANSI/SBS 4-2004 standard for 96-well titer plates
- Volume: 243µl ± 5µl
- Bottom Diameter: 5.0mm ± 0.05mm
- Electrical Interface: interface with 96x E-Plate device station
- Sensor Impedance: 17ohm ± 5ohm @ 10kHz, when measured with a 1X PBS Solution
- Material: biocompatible surfaces
- Sterile
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

16x RT-CIM Device Lower Chamber

- Dimension: 75 mm L x 40 mm W x 7.5 mm H

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- Spacing: The spacing of the wells is to be 9mm center-to-center per the ANSI/SBS 4-2004 standard for 96-well titer plates
- Volume: 129 µl ± 5µl (152 µl ± 5µl, if including the gasket on the bottom of the top chamber)
- Bottom Diameter: 5.0mm ± 0.05mm
- Mechanical Assembly: assembled with RT-CIM upper chambers
- Material: biocompatible surfaces
- Sterile
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

16x RT-CIM Device Upper Chamber

- Dimension: 86.5 mm L x 40 mm W x 9.5 mm H (with plate cover)
- Spacing: The spacing of the wells is to be 9mm center-to-center per the ANSI/SBS 4-2004 standard for 96-well titer plates
- Volume: 140 µl ± 5µl
- Bottom Diameter: 4.0mm ± 0.05mm
- Electrical Interface: contact pads with POGO pins on 16x RT-CIM device station
- Mechanical Assembly: assembled with RT-CIM lower chambers
- Sensor Impedance: 36 ohm ± 16 ohm @ 10kHz, when measured with a 1X PBS Solution
- Material: biocompatible surfaces
- Sterile
- Environment: 15˚C to 40˚C, Relative Humidity: 98% max non-condensing

16x RT-CES Resistor Board

- Resistors: 16 resistors of four different values, when measured with RT-CES system, showing well resistances averaged between 12 – 17 ohms @10 kHz with standard deviation less than 5%
- Electrical Interface: interface with 16x RT-CES E-Plate station

96x RT-CES Resistor Plate

- Resistors: 96 resistors of four different values, when measured with RT-CES system, showing well resistances averaged between 12 – 17 ohms @10 kHz with standard deviation less than 5%
- Electrical Interface: interface with 96x RT-CES E-Plate station

16x RT-CIM Resistor Board

- Resistors: 16 resistors of four different values, when measured with RT-CES system, showing well resistances averaged between 12 – 17 ohms @10 kHz with standard deviation less than 5%
- Electrical Interface: interface with 16x RT-CIM station

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Installation

Installation Warnings

1. Always make sure that the power switch on the RT-CES Analyzer (and, if applicable, MultE-Plate station) is in the OFF position, and remove the power cord from the back of the instrument prior to installing or relocating the instrument.
2. Do not operate the instrument in an environment where potentially damaging liquids or gases are present.
3. Connect the RT-CES analyzer (and, if applicable, MultE-Plate station) to a grounded AC supply line using the supplied chord. Failure to observe the installation instruction may increase the risk of electrical shock and/or fire, and/or may affect the performance of the RT-CES system.
4. The RT-CES Analyzer must be placed in a location that allows easy access to the rear panel On/Off Power Switch.
5. Do not place anything within 4 inches (100 mm) of the side ventilation ports of the Analyzer. Failure to observe this may result in overheating of the equipment and increase the risk of fire.
6. After installing an E-Plate Station and/or a RT-CIM station in an incubator, allow at least two hours before attempting operation. This allows the Station to reach equilibrium with the incubator environment and reduces the risk of condensation-related problems.

Installation Cautions

Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might affect instrument performance and void the instrument warranty.

Unpacking

The RT-CES analyzer and E-Plate stations (and RT-CIM stations) are packed in a specially designed carton. Retain the carton and packing materials. If the system needs to be returned for repair, you must use the original packing materials and carton for shipping. If the carton has been damaged in transit, *retain it for inspection by the carrier in case there has also been damage to the instrument.*

Warning:

> ***The RT-CES analyzer weighs approximately 15 lbs (7.8 kg) and should be lifted with care. It is recommended that two people lift the instrument together, taking proper precautions to avoid injury.***
>
> ***The MultE-Plate station weighs approximately 18 lbs (16 kg) and should be lifted with care. It is recommended that two people lift the instrument together, taking proper precautions to avoid injury.***

After examining the carton, place it on a flat surface in the upright position. Open the top of the box and lift the RT-CES Analyzer, along with the packing materials around the edges, up and out of the shipping box. Remove the packing materials from the ends of the instrument and set the instrument down carefully.

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Setting up the RT-CES system or RT-CIM system

Set up the computer (RT-CES SP or MP software is pre-installed)

1. Remove all components from cartons containing the computer system. Detailed instructions for the computer system are included.
2. Attach the power cord, mouse, and keyboard to the computer system.
3. Attach the power cord and monitor cable to the monitor, and attach the other end of the monitor cable to the computer using a splitter converter cable (a short cable with a single plug leading to 2 plugs) or adaptor, as needed.
4. Plug the computer system into the wall outlet (it is recommended that you use a surge protector power strip) along with the monitor power cord.
5. Power on the computer and the monitor.
6. ACEA RT-CES SP or MP Software is pre-installed on the system and displays on the desktop as an ACEA RT-CES icon, .The description of the icon is “ACEA RT-CES SP v#.#”, or “ACEA RT-CES MP v#.#”.
7. Click the icon to open the ACEA RT-CES SP software. The ***RT-CES SP / MP Software User’s Manual*** is in the Analyzer box.

RT-CES SP or MP software repair / reinstallation

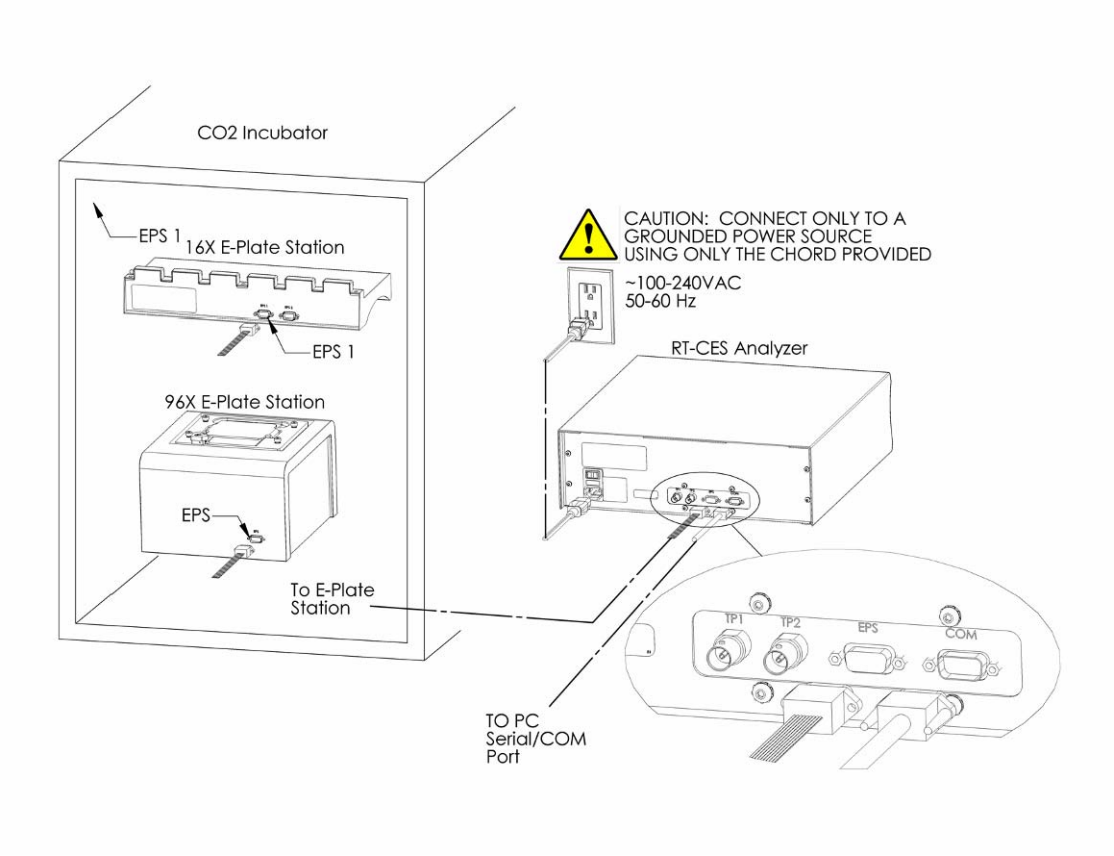
RT-CES SP or MP software is pre-installed on the computer which is shipped to the customer from ACEA. The attached software CD is for repairing / reinstallation purpose. In case the computer is replaced, or reinstalled Windows, or for whatever reasons the RT-CES does not work properly, RT-CES SP or MP repair / reinstallation is needed.

To repairing / reinstallation:

1. Locate the CD containing the RT-CES SP or MP software.
2. Put the CD into the CD drive and wait for several seconds. The installation program will start automatically.
3. (if the installation program does not start automatically) Open **My Computer** on your PC and double-click the CD drive. Note the RT-CES Icon labeled RT-CES SP v #. #, or RT-CES MP v#.#. After the details of the CD are shown, double click on “Setup.exe” to start the installation program.
4. After the installation starts, if RT-CES is already installed, the installation program will ask for “Repair” or “Reinstallation”. Choose either of the options to continue. Follow the instructions of the installation program. If the customer accepts all the default settings, just click “Next”, “Next” to finished the installation.
5. After the installation, the  icon is on the desktop, as well as in the Windows programs menu.
6. Double click the  icon to launch the application.

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Set up RT-CES Analyzer and E-Plate Station (16x or 96x)



Remove instruments from boxes

1. Remove RT-CES Analyzer from the box along with the RT-CES User Guide.
2. Remove E-Plate Device Station from the shipping box.

Connect the RT-CES Analyzer to the computer

3. Locate the serial cable that has two female connector ends (9-pin). Plug one end of the cable into the COM port of the computer and attach the other end of the cable to the COM port on RT-CES Analyzer.

Place the E-Plate Stations (16x or 96x) into an incubator

4. Wipe the E-Plate Station with 70% alcohol or another sterilizing agent.
5. Place the E-Plate Station into the incubator and run the ribbon cable (flat black cable) from the E-Plate Station to outside of the incubator, making sure that the incubator door closes. If round grey cable used, please have the round grey cable go through ACCESS PORT typically located on the back or a side of the incubator.

Connect the E-Plate stations to the RT-CES Analyzer

6. Insert the male connector at the end of the ribbon cable to the EPS port on the rear panel of the RT-CES Analyzer.

Note: It is important to wait for at least 2 hrs (after placing the E-Plate station inside the incubator) before connecting the E-Plate stations to the RT-CES Analyzer.

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Attach the power cord to RT-CES Analyzer

7. Attach the power cord to the Analyzer and plug it into the wall outlet.
8. Turn the power switch, located on the back of the Analyzer, to "ON."

The front panel display of the analyzer reads:

READY

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The Analyzer and the E-Plate Station are ready to run an assay.

Note:

1. *The instrument is pre-programmed and ready to communicate with the RT-CES software.*
2. *Do not press the key buttons on the front panel. If you have depressed any of the front panel buttons, use "Reset" to set the instrument to its default settings.*
3. *It is important to wait for at least 2 hrs (after placing the E-Plate station inside the incubator) before turning on the power switch of the RT-CES Analyzer.*

Set up RT-CES Analyzer and 16x RT-CIM Station

Remove instruments from boxes

1. Remove RT-CES Analyzer from the box along with the RT-CES User Guide.
2. Remove 16x RT-CIM Station from the shipping box.

Connect the RT-CES Analyzer to the computer

3. Locate the serial cable that has two female connector ends (9-pin). Plug one end of the cable into the COM port of the computer and attach the other end of the cable to the COM port on RT-CES Analyzer.

Place the 16x RT-CIM Stations into an incubator

4. Wipe the 16x RT-CIM Station with 70 % alcohol or another sterilizing agent.
5. Place the 16x RT-CIM Station into the incubator and run the ribbon cable (flat black cable) from the E-Plate Station to outside of the incubator, making sure that the incubator door closes. If round grey cable used, please have the round grey cable go through ACCESS PORT typically located on the back or a side of the incubator.

Connect the 16x RT-CIM stations to the RT-CES Analyzer

6. Insert the male connector at the end of the ribbon cable to the EPS port on the rear panel of the RT-CES Analyzer.

Note: It is important to wait for at least 2 hrs (after placing the 16x RT-CIM station inside the incubator) before connecting the 16x RT-CIM stations to the RT-CES Analyzer.

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Attach the power cord to RT-CES Analyzer

7. Attach the power cord to the Analyzer and plug it into the wall outlet.
8. Turn the power switch, located on the back of the Analyzer, to "ON."

The front panel display of the analyzer reads:

READY

ACEA BIOSCIENCES INC

The Analyzer and the 16x RT-CIM Station are ready to run an assay.

Note:

1. The instrument is pre-programmed and ready to communicate with the RT-CES software.

2. Do not press the key buttons on the front panel. If you have depressed any of the front panel buttons, use "Reset" to set the instrument to its default settings.

3. It is important to wait for at least 2 hrs (after placing the 16x RT-CIM station inside the incubator) before turning on the power switch of the RT-CES Analyzer.

Set up RT-CES Analyzer and MultE-Plate (MP) Station

Remove instruments from boxes

1. Remove RT-CES Analyzer from the box along with the RT-CES User Guide.
2. Remove MultE-Plate Station from the shipping box.

Connect the RT-CES analyzer to the computer

3. Locate the serial cable that has two female connector ends (9-pin). Plug one end of the cable into the COM port of the computer and attach the other end of the cable to the COM port on RT-CES Analyzer.

Place the MP station into an incubator

4. Wipe MP Station with 70% alcohol or another sterilizing agent.
5. Place MP Station into the incubator. (MP station is rather heavy and please take care during this process. It just fits inside the incubator. Cares should be taken to avoid scratching of the MP station chassis by the interior parts of the incubator. Two people are recommended in placing MP station into the incubator).
6. There are three cables/wires that are connected to MP Station: a round grey cable with DB9 (male) and Lemo connector (12 connections) between RT-CES analyzer and MP station, a round grey cable with DB9 (female) and Lemo connector (8 connections) between a computer port and MP station, and a DC power plug to MP station from an AC adaptor. The three cables/wires should go into the incubator through an access port typically located at the back or the side of an incubator with Lemo connectors (12 and 8 connections) and the DC power plug inside the incubator.

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Connect the Power cord to the MP station

7. Attach the DC adaptor (with attached power cord) to MP station and plug the power cord to an AC outlet. DO NOT TURN ON the MP station.

Connect the MP station to the RT-CES Analyzer

8. Connect analyzer to MP station with the grey round cable having DB9 (male, for analyzer) and Lemo connector (12 connections, 6 pins and 6 holes, for MP station). Make sure that the Lemo connector is oriented correctly to the socket on the MP station. Hold the outmost metal cover of the Lemo connector and gently push into the socket. Do not force the Lemo connector in. Otherwise, it will damage the connector. Similarly, when you want to unplug the Lemo connector, hold the outmost metal cover on the connector, gently pull the connector. Do not pull the cable out. The metal cover is slightly movable with respect to the cable and that's how the connector can be fully engaged onto the socket. Directly pulling the cable will result in the damage of the connector and the cable.

Connect the MP station to the Computer

9. Connect a computer USB port (For Dell Laptop, we recommend to use the USB port at the back, top right port of the computer when the computer monitor is facing the operator) to the MP station using the USB-to-Serial Port converter and the DB9 (female) to Lemo connector (8 connections, 4 pins and 4 holes). Make sure that the Lemo connector is oriented correctly to the socket on the MP station. Hold the metal cover of the Lemo connector and gently push into the socket. Do not force the Lemo connector in. Otherwise, it will damage the connector. Similarly, when you want to unplug the Lemo connector, hold the outmost metal cover on the connector, gently pull the connector. Do not pull the cable out. The outmost metal cover is slightly movable with respect to the cable and that's how the connector can be fully engaged onto the socket. Directly pulling the cable will result in the damage of the connector and the cable.

Attach the power cord to RT-CES Analyzer and computer

10. Attach the DC power plug of the AC adaptor (with attached power cord) to Laptop computer and connect the power cord to an AC outlet. DO NOT TURN ON the computer.
11. Attach the power cord to the Analyzer and plug it into an AC outlet. DO NOT TURN ON analyzer.
12. Turn on the computer.

    The PC to run MP needs 2 COM ports. One connects to Analyzer for data collection, the other to MP Station for motor control. If the PC does not have 2 COM ports, the USB-to-Serial Port converter is used (as mentioned above in "**Connect the MP station to the Computer"** paragraph**).**
13. Since different USB slots may be allocated to different COM port numbers, make sure which COM port(s) are available at the PC before starting experiment or running the MP software.

To check what COM port(s) available, follow the steps shown below.

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1) Right Click on **My Computer** icon on the desktop; Select **Properties**

2) Select **Hardware** Tab and then click on **Device Manager**

3) Click on **Ports (COM & LPT)**, then the available COM ports are shown (for example, it would indicate: USB serial port (COM4)). The port number is needed for RT-CES MP software to communicate properly with MP station. Please refer to RT-CES SP/MP Software User's Manual as to how to set or modify the communication port number for motor control of MP station.

14. Turn on the analyzer (the power switch is located on the back of the Analyzer). The front panel display of the analyzer reads:

READY

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15. Turn on MP station (the power switch is located to the right of the front keypad panel). The Power LED should be ON and motor-status LEDs should indicate the UP or DOWN position of each motor module. There are six motor modules (or plate positions) in two rows. The positions in the bottom row are 1, 2 and 3, counting from left and the positions in the top row are 4, 5 and 6, counting from left. You can use the Keypad to Move up or Down the motor module (plate holder). At any moment, only one motor can be activated.

    For example, you can Move UP the Position 1. Put a 96x resistor board on the Plate Pocket on Position 1. Move Down the Position 1.

The Analyzer and the MP-Plate Station are ready to run an assay.

Note:

1. *Both analyzer and MP-station are pre-programmed and ready to communicate with the RT-CES MP software.*
2. *Do not press the key buttons on the analyzer front panel. If you have depressed any of the front panel buttons, use "Reset" to set the instrument to its default settings.*
3. *It is important to wait for at least 2 hrs (after placing the E-Plate station inside the incubator) before turning on the power switch of the RT-CES Analyzer.*

Resistor Board Verification -- for 16x E-Plate Station

Insert resistor board

1. Insert the six 16x Resistor Boards into the ZIF connector sockets (20-holes each) on the 16x E-Plate Station. Lock the Resistor Board to the connector by pushing the small lever located on the socket 90 degrees to the RIGHT. If connected properly, the green light turns on.

Note:

1. *Make sure that all the twenty connector pins of the 16x Resistor Board are straight and parallel to each other. If not, use finger or tweezers to straighten the "bent" connector pin. Failing in doing may lead to unreliable connection between the ZIF socket and the Resistor Board.*

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2. *Make sure that all the twenty connector pins of the 16x Resistor Board are clean and free from any dust or dirty particles. If not, wipe the connector pins with 70% alcohol and/or dust-off the connector pins with compressed air.*
3. *Make sure that all the twenty connector pins of the 16x Resistor Board are properly aligned with the holes in the ZIF socket. If all the pins are straight and if all the pins are properly aligned with the ZIF socket, There is no need to push or force the 16x Resistor Board into the ZIF socket. Forcing the connectors into the socket may damage the interior surface of the ZIF socket, leading to shortened life time for the ZIF socket.*
4. *Make sure that before inserting the 16x Resistor Board, the small lever on the station is oriented parallel with the connector pins on the Resistor Board.*

Start the RT-CES SP program

1. Start the RT-CES SP program by double-clicking the program icon  on the desktop. If the program is already open, close it and then reopen it.
2. Setup the ExpNote, Layout (e.g. turning on all the wells), and Test Time Pages (e.g., step one, 5 sweeps, one minute interval). For more information about setting up the **Test Time** Page, see RT-CES SP/MP software user's manual.

Perform the test

1. Click **Start** to begin the test. While the test is running, notice the bottom of the program window reads: "**testing column 1, 2, ….12**", and the RT-CES analyzer displays the message: "**Measurement in Progress…….**"

Check the data

1. Upon completion of the test, check the Cell Index Page. All of the Cell Index values should be less than 0.03. In addition, go to the data file folder. The folder name is in the following format: mmddyyhhmm (i.e., month-date-year-hour-minute the experiment was started). Open the calibration.txt file. There are three groups of data, each group having 8 rows by 12 columns. All of the data in the last group should have values between 13 and 17.

Remove the resistor boards

1. To remove the 16x resistor boards, gently pull the lever on the connector socket forward. The green light turns off. Slide the pins of the resistor boards out of the socket and remove the device.

Resistor Board Verification -- for 96x E-Plate Station

Place the 96x resistor board plate on the station

1. Insert one 96x Resistor Board Plate (with plate cover) into the slot on the 96x E-Plate Station. Ensure that the angled corners are inserted first and the plate is fully inside the pocket. Push the plate away from you twice, so that the spring on the top-left corner resets the plate against the pocket edge.
2. Press down the front handle with one hand, while holding the box near the front with the other hand, until it latches into place. The handle clicks into place ***Please refer to the Procedure for pressing down the front handle for more information.***

Note:

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1. *Make sure that all the 120 connector pads of the 96x Resistor Board are clean and free from any dust or dirty particles. If not, wipe the connector pins with 70% alcohol and/or dust-off the connector pins with compressed air.*
2. *When inserting the 96X Resistor Board into the slot on the Station, make sure that the Resistor Board Plate is FULLY inside the pocket and is aligned properly with the edges of the pocket.*
3. *The green light on the front panel of the E-plate station is on as long as the E-Plate station is connected to the analyzer, which is turned on.*

Start the RT-CES SP program

1. Start the RT-CES SP program by double clicking the RT-CES SP program  icon on the desktop. If the program is already open, close it and then reopen it.
2. Set up the ExpNote, Layout (e.g. turning on all the wells), and Test Time Pages (e.g., step one, 5 sweeps, one minute interval). For more information about setting up the **Test Time** Page, see RT-CES SP/MP software user's manual.

Perform the test

1. Click **Start** to begin the test. While the test is running, notice the bottom of the program window reads "**testing column 1,2,3, ….12**", and the RT-CES Analyzer displays the message: "**Measurement in Progress…….**"

Check the data

1. Upon completion of the test, check the Cell Index Page. All of the Cell Index values should be less than 0.03. In addition, go to the data file folder. The folder name is in the following format: mmddyyhhmm (i.e., month-date-year-hour-minute the experiment was started). Open the calibration.txt file. There are three groups of data, each group having 8 rows by 12 columns. All of the data in the last group should have values between 13 and 17.

Remove the Resistor Boards

1. To remove the 96X resistor board, press down lightly on the handle, depress the red button beneath the handle, and slowly allow handle to slide up. Slide the resistor board out, toward you. ***Refer to the Procedure for pressing down the front handle section for detailed instructions about releasing the front handle of the 96X E-Plate station.***

Resistor Board Verification -- for 16x RT-CIM Station

Place the 16x RT-CIM resistor board plates on the station

1. Place a 16x RT-CIM Resistor Board Plate into each of the six modules on the 16x RT-CIM Station. Total six resistor boards are placed into the six modules.
2. With the release-button pressed-down, close the module cover. Let go the release button to lock down each RT-CIM module**.**

Note:

1. Make sure that all the 20 connector pads on each of the 16x CIM Resistor Board are clean and free from any dust or dirty particles. If not, wipe the

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connector pads with 70% alcohol and/or dust-off the connector pads with compressed air.

2. When placing a 16X RT-CIM Resistor Board into a module on the Station, make sure that the 16X Resistor Board Plate is positioned flat inside the module and aligned properly with the edges of the module.

3. The green light on the front panel of the 16x RT-CIM station is on as long as the 16x RT-CIM station is connected to the analyzer, which is turned on.

Start the RT-CES SP program

1. Start the RT-CES SP program by double clicking the RT-CES SP program  icon on the computer. If the program is already open, close it and then reopen it.
2. Set up the ExpNote, Layout (e.g. turning on all the wells), and Test Time Pages (e.g., step one, 5 sweeps, one minute interval). For more information about setting up the **Test Time** Page, see RT-CES SP/MP software user's manual.

Perform the test

1. Click **Start** to begin the test. While the test is running, notice the bottom of the program window reads "**testing column 1,2,3, ….12**", and the RT-CES Analyzer displays the message: "**Measurement in Progress…….**"

Check the data

1. Upon completion of the test, check the Cell Index Page. All of the Cell Index values should be less than 0.03. In addition, go to the data file folder. The folder name is in the following format: mmddyyhhmm (i.e., month-date-year-hour-minute the experiment was started). Open the calibration.txt file. There are three groups of data, each group having 8 rows by 12 columns. All of the data in the last group should have values between 13 and 17.

Remove the 16x RT-CIM Resistor Boards

1. To remove the 16X RT-CIM resistor board, gently press down on the release button and open the cover of the module. Remove the resistor board from the module.

Resistor Board Verification -- for 96x MP Station

Note: In this example, two 96x resistor board plates are used to verify the operation for the two plate positions (plate 1: EP1 and plate 2: EP2).

Place the 96x resistor board plates on the station

1. Press EP1 "Up" button (Δ) on the front panel of MP station to move motor module 1 to its "Top" position. Insert one 96x Resistor Board Plate (with plate cover) into module 1 of the MP station. Ensure that the angled corners are inserted first and the plate is fully inside the pocket.

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2. Press EP 1 "Down (∇)" button on the front panel of the MP station to move the resistor plate on its test position (bottom).

3. Repeat steps 1 and 2 for EP2 for placing and engaging a second resistor board plate for module 2.

Note:

1. Make sure that all the 120 connector pads of the 96x Resistor Board are clean and free from any dust or dirty particles. If not, wipe the connector pins with 70% alcohol and/or dust-off the connector pins with compressed air.

2. When inserting the 96X Resistor Board into the slot on the Station, make sure that the Resistor Board Plate is FULLY inside the pocket and is aligned properly with the edges of the pocket.

Start the RT-CES MP program

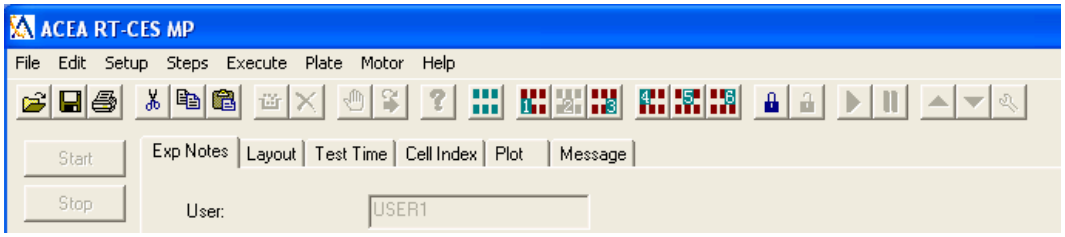
1. Start the RT-CES MP program by double clicking the ACEA RT-CES MP program icon on the desktop (if the program is already open, close it and reopen the program).

Login the RT-CES MP program

1. When RT-CES MP program is launched, Login Window is shown. If RT-CES MP program is running and logout, a user needs to click on "Login" button to active login window. Enter user name and password, and then click on "Enter" to login to RT-CES MP program.

Select a Plate (1~6)

1. Select a plate by click on the plate icon located on the top-right. In the image below, Plate 2 is selected.



Select Plate (Plate 2 is selected)

Setup ExpNote Page, Layout Page, Test Time Page

With Selected Plate being Plate 1, setup ExpNote/Layout/Test Time Pages.

1. Setup **ExpNote** Page. This page records experiment-related information for Selected Plate such as User, Experiment Date, Experiment Name, Experimental

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Purpose, Experimental Procedure, Experimental Conclusion and the Devices you used in this experiment. Simply type in any information you like to record into appropriate spaces. The information will be saved when the experiment is started. You can also use BROWSE to choose a File Directory into which the experimental files will be saved. Further details about setup **ExpNote** Page are provided in RT-CES SP/MP software user's manual. Note that **ExpNote** Page in RT-CES MP software is used in a similar way to **ExpNote** Page in SP software.

2. Setup **Layout** Page. This Page shows the Experiment Layout and records experiment details about each well, including name and number of cells, name and concentration of compounds added into wells. Activate all the 96 wells with Cell Type being set as R or Resistors. Details about setup **Layout** Page are provided in RT-CES SP/MP software user's manual. Note that **Layout** Page in RT-CES MP software is used in a similar way to **Layout** Page in SP software.

3. Setup **Test Time** Page. This page is used to program the experimental procedure. Generally, experiment can be divided into multiple steps, and each step consists of one or multiple sweeps. For Resistor Board verification here, just add Step 1 with 5 sweeps, 1 minute for the time interval. Further details for setup the **Test Time** Page are shown in RT-CES SP/MP software user's manual. Note that **TestTime** Page in RT-CES MP software is used in a similar way to **TestTime** Page in SP software.

After setup ExpeNote/Layout/Test Time Pages for Plate 1, select Plate 2 and follow above procedure to setup ExpeNote/Layout/Test Time Pages for Plate 2.

Perform the test

1. Go to Motor control and Plate Status Page. Click **Start All** to begin the test. While the test is running, notice the bottom of the program window reads "**Plate # test column # Sweep # Step #**", and the RT-CES Analyzer displays the message: "**Measurement in Progress…….**", where # is a number referring to plate number, or column number, or sweep number or step number. For the Resistor Board test here, MP system will perform tests alternatively between Plate 1 and Plate 2.

Check the data

1. Upon completion of the test, check the Cell Index Page for Plate 1 and Plate 2 separately by selecting Plate 1 and Plate 2, respectively. All of the Cell Index values should be less than 0.03. In addition, go to the data file folder. The folder name is in the following format: mmddyyhhmmP# (i.e., month-date-year-hour-minute the experiment was started, with P1, P2, … or P6 added at the end of the file name). Open the calibration.txt file. There are three groups of data, each group having 8 rows by 12 columns. All of the data in the last group should have values between 13 and 17.

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Remove the Resistor Boards

1. To remove the 96X resistor board, click UP button on the MP status page or press the UP (Δ) button on the front panel of the MP station. Slide the resistor board out, toward you.

Exchange between 16x and 96x E-Plate Stations

If you have both 16x and 96x E-Plate Stations and need to switch from a current station (e.g. 16x/96x Station) to a new station (e.g., 96x/16x Station), follow the instructions below. (Special Note: If you have a RT-CIM station and a 16x or 96x E-Plate Station, following instructions can also be applied for exchange between RT-CIM Station and 16x/96x E-Plate Station.

Turn off the power to RT-CES Analyzer and remove the current station

1. Turn off the power to the RT-CES Analyzer. Unplug the attached AC power cord from the power outlet.
2. Detach the ribbon cable, which is attached to the current E-Plate Station and to the RT-CES analyzer, from the EPS port of the RT-CES Analyzer.
3. Remove the current E-Plate Station from the incubator.

Place a new E-Plate Station in the incubator

1. Wipe the E-Plate Station with 70% alcohol or another sterilizing agent.
2. Place the E-Plate Station into the incubator and run the ribbon cable (flat grey and red cable) from the E-Plate Station to outside of the incubator, making sure that the incubator door closes.

Connect the new E-Plate station to the RT-CES Analyzer

1. Insert the connector at the end of the ribbon cable to the EPS port on the rear panel of the RT-CES Analyzer.

Attach the power cord to RT-CES Analyzer

1. Attach the power cord to the RT-CES Analyzer and plug it into a wall outlet.
2. Turn the power switch, located on the rear panel of the Analyzer, to “ON.”
3. The front panel of the analyzer displays:
   READY

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   The RT-CES Analyzer and the E-Plate Station are ready to run an assay.

Note:

1. ***When not in use, the E-Plate Stations (16x or 96x or MultE-Plate) should be placed in a clean, sealed package (for example, in the original plastic bag(s) when the Stations were shipped). This is important for the Stations to have consistent, reliable connections***

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to the E-Plates and also important for the Stations to have a long, functional life.

2. ***Each time when the E-Plate Station is used after a long time storage, use compressed air or N2 to dust-off the holes in the six ZIF sockets of a 16x E-Plate Station, or to dust-off the POGO-pins on a 96x or a MultE-Plate Station. Make a visual inspection to make sure that all the POGO pins are standing upright and are about the same height.***
3. ***If any POGO pin is visually shorter than others, try to Reset the POGO-pin using a flat-head screw driver to push down the POGO pin and then let it go. Repeat this "Push down and let it go" a couple of times. The POGO-pin will return to its normal height. If not, contact ACEA Biosciences.***
4. ***If a POGO pin is visually not standing upright, please contact ACEA Biosciences.***

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Procedure for pressing down the front handle on a 96x station

1. Starting position.



2. Press the red button all the way in.



3. While pushing the red button in, lower the front handle all the way.



4. While the handle is lowered, release the red button.



5. Release the handle. The handle will move up slightly, but it is locked in position.



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Procedure for releasing the front handle on a 96x Station

1. Starting position.



2. Press down on the front handle, pressing it to the lowest position.



3. While the handle is pressed down, push the red button all the way in.



4. While pressing the red button in, release the front handle. The handle moves up.



5. Release the red button. The handle stops at its highest position.



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System Operation

Running a quick RT-CES experiment

Note: Before running this quick experiment, ensure that all the following items are checked:

1. Ensure that the RT-CES System is properly installed and that the two cable connections (between the computer and RT-CES Analyzer, and between the RT-CES Analyzer and E-Plate Station) are properly linked.
2. During the check, if connections are redone, perform the resistor board verification again (see Resistor Board Verification -- for 16x E-Plate Station and/or Resistor Board Verification -- for 96x E-Plate Station for more information).
   In addition, if the system has not been used for over a month, perform resistor board verification.
3. When the RT-CES analyzer is turned on. The front panel display reads:

   READY

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Note: If this is not what displays on the Analyzer, press the RESET button.

This cell titration experiment being run on a 16x E-Plate device is used for illustration purpose. The procedure for this experiment includes:

- Adding culture media to the 16X E-Plate
- Inserting the 16X E-Plate
- Starting the RT-CES SP software program
- Setting up ExpNote, Layout, and Test Time pages
- Starting the experiment
- Removing the 16x E-Plate and adding cells into the E-Plate wells
- Reinserting the 16X E-Plate
- Checking and plotting the Cell Index data

Add Culture Media to the 16X E-Plate

1. Add 50 or 100 µL of cell culture media into each of 16 wells in the 16X E-Plate. For optimal results, leave the E-Plate in the tissue culture hood for 15 minutes at room temperature. This ensures that the culture media and E-Plate surface achieve equilibrium.

Note: The 16 wells (or 96 wells) within 16X (or 96X) E-Plates are organized so that each column of 8 wells is divided into two 4-well groups. During the experiment, to achieve consistent and accurate data, it is necessary to use the wells within a group in the same experiment. Examples of such 4-well groups are: A1, B1, C1 and D1 as a group; E3, F3, G3 and H3 as a group, A7, B7, C7, D7 as a group, and so on.

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Insert the 16X E-Plate

1. Insert the 16x E-Plate into one of the ZIF connector sockets (20-holes each) on the 16x E-Plate Station.
2. Lock the E-Plate to the connector by pushing the small lever located on the socket 90 degrees to the right. The green light turns on when it is connected properly.
3. Close the door of the incubator.

Note:

1. *Make sure that all the twenty connector pins of the 16x E-Plate are straight and parallel to each other. If not, use finger or tweezers to straighten the "bent" connector pin. Failing in doing may lead to unreliable connection between the ZIF socket and the Resistor Board.*
2. *Make sure that all the twenty connector pins of the 16x E-Plate are clean and free from any dust or dirty particles. If not, wipe the connector pins with 70% alcohol and/or dust-off the connector pins with compressed air.*
3. *Make sure that all the twenty connector pins of the 16x E-Plate are properly aligned with the holes in the ZIF socket. If all the pins are straight and if all the pins are properly aligned with the ZIF socket, There is no need to push or force the 16x E-Plate into the ZIF socket. Forcing the connectors into the socket may damage the interior surface of the ZIF socket, leading to shortened life time for the ZIF socket.*
4. *Make sure that before inserting the 16x E-Plate, the small lever on the station is oriented parallel with the connector pins on the Resistor Board.*

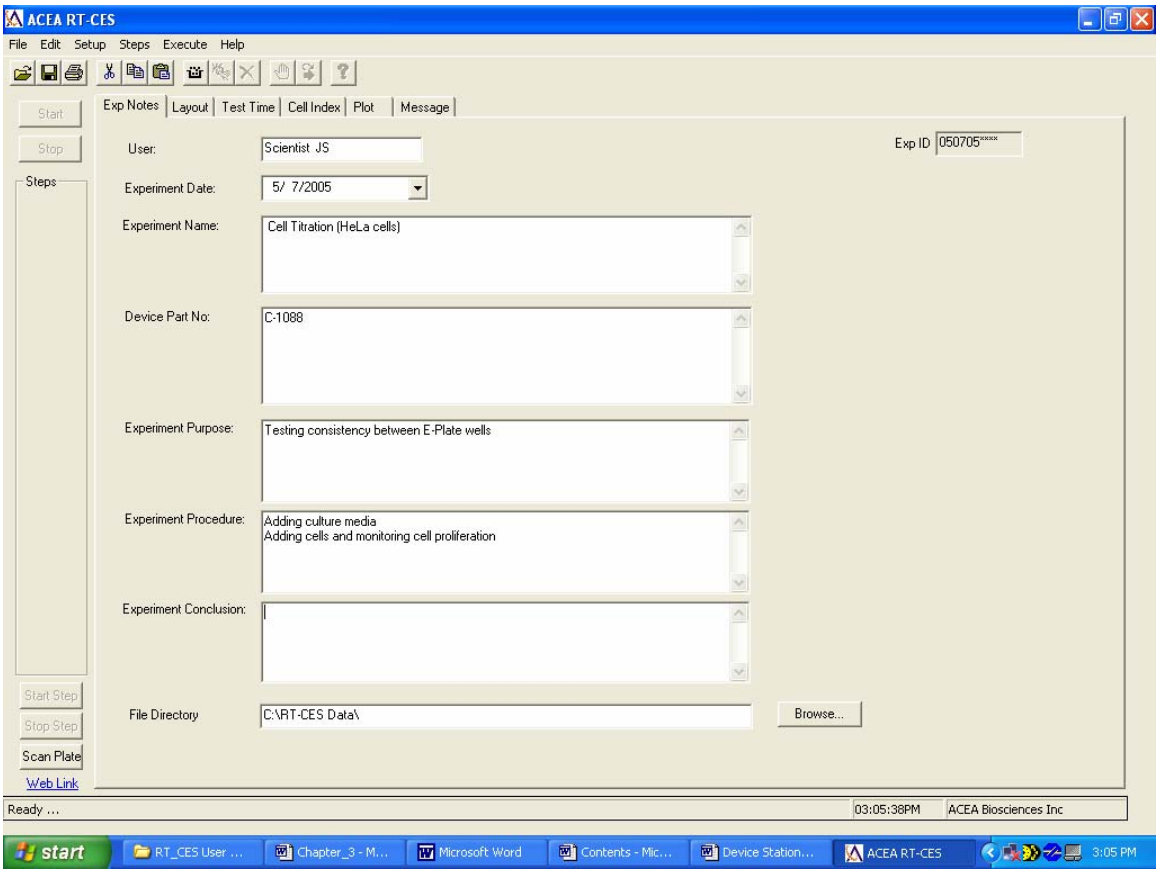
Start the RT-CES SP program

1. Start the RT-CES SP software program by double clicking the ACEA RT-CES SP icon on the desktop (if the program is already open, close it, and then reopen it).

Set up the ExpNote, Layout, and Test Time pages

1. Set up the **ExpNote** page. This page records experiment-related information such as user, experiment date, experiment name, experimental purpose, experimental procedure, experimental conclusion, and the devices used in the experiment. Enter the information to record in the appropriate spaces. When the experiment starts, this information is automatically saved. You can also click **BROWSE** to select a File Directory to save the experiment files to. See **RT-CES SP/MP Software User's Manual** for more information about setting up the **EXPNote** page.

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Sample Experiment Note Page

2. Set up the **Layout** page. This page determines the experiment layout and records experiment details about each well, including: name and the number of cells, name and concentration of compounds added into wells, etc.

Select the wells to be edited:

a. To select a single well, hover over the well to be selected, and left-click. The selected well is highlighted. The well ID of the selected well, is automatically filled into the Well IDs section. If, for example, the well D1 is selected, then the Well IDs section reads:" D1:D1".

b. Enter the ID of the selected well directly into the Well IDs Box. If, for example, B2 is selected, enter B2:B2 into the Well IDs section. Or, if all wells between A1 and H2 are selected, type in A1:H2 into Well IDs section. The selected well is highlighted when the mouse moves from the Well IDs section to another section, such as Cell Type.

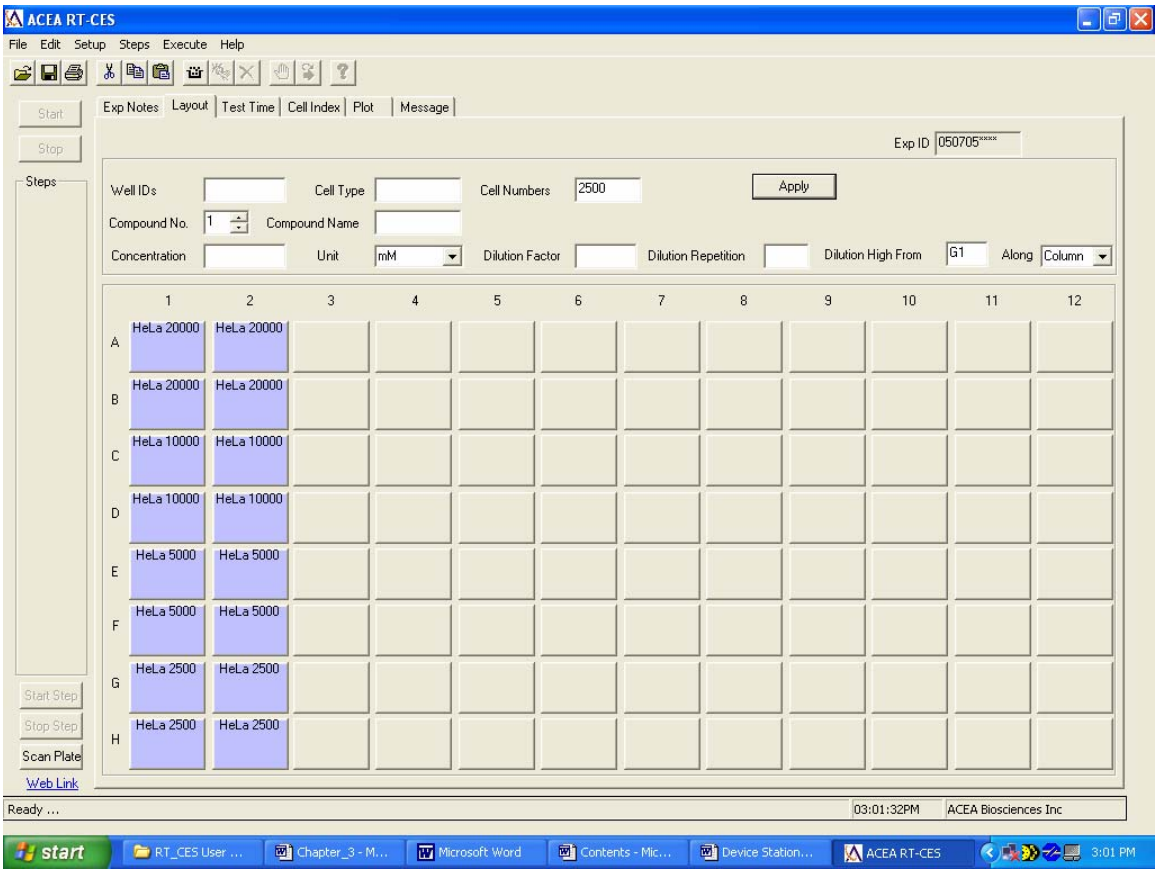
c. To select multiple wells, hover over the first well to be selected, left-click and hold the mouse. Drag the mouse across the other wells, to the last well to be selected. Release the mouse button. All selected wells are highlighted, the well's ID, for example A1:D2, is automatically filled into the Well IDs section, if all wells between A1 and D2 are selected. Note that the text in the Well IDs section has the format *First well ID: Last well ID*.

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d. To select an entire row(s) or column(s), move the mouse to the row ID (e.g. A or E) or column ID (e.g. 1 or 3), and left-click. To select adjacent multiple-rows or multiple columns, left-click and drag across row IDs or column IDs.

Enter information into appropriate information edit boxes, and click **APPLY**. For example: Cell Type – MDA-231; Cell Number – 5000.

The other fields, including Compound No., Compound Name, Concentration, Unit, Dilution Factor, Dilution Repetition, Dilution High From, Along *Column or Row* is described in the **RT-CES SP/MP Software User's Manual**.



Sample Experiment Layout page

3. Set up the **Test Time** page. This is used to program the experimental procedure. Generally, experiment can be divided into multiple steps, and each step consists of one or multiple sweeps.

Note: Each sweep is one measurement across all the selected wells. There is a pre-determined time interval between neighboring sweeps within a step. After completing a current sweep, the RT-CES system automatically starts to count down the time interval and starts the next sweep.

After completing all of the sweeps within a step, the RT-CES system does not move on to the next step until it is manually instructed to do so. There is always a manual intervention for the system to move from one step to the next one. Generally, each time an E-Plate is removed, there should be a separate step. Users can manually stop a current step before the system completes all pre-set sweeps. However, a current

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step cannot be manually stopped in the middle of a measurement sweep. Doing so may cause system instability and data loss.

Step 1 is typically used for background tests, where no cells are present in the wells. It is typically set as One sweep, One minute. To add Step 1:

a. Move the mouse to the **Steps** column on the left side of the page.

b. Left click the Add a Step Icon , or go to the pull-down Steps menu and select Add a Step. Step 1 is added.

The Step Status is idle, and all other buttons are blank. Edit the Intervals and Sweeps section by entering the appropriate numbers. Select the Interval Units in hours or in minutes (or in seconds for RT-CES SP software with version No, 5.2 or higher). Click **Apply**, and the appropriate time intervals, with a unit and sweeps are added. The Sweep Status is automatically set to 0. Test Status displays as Test while the experiment is in progress. When the step is completed, Done displays. The Sweep Status section is updated and displays the number of sweeps that have been completed.

Note:

For RT-CES SP 5.2 or above, the sweep number and sweep interval for the Step 1 is pre-set by the software.

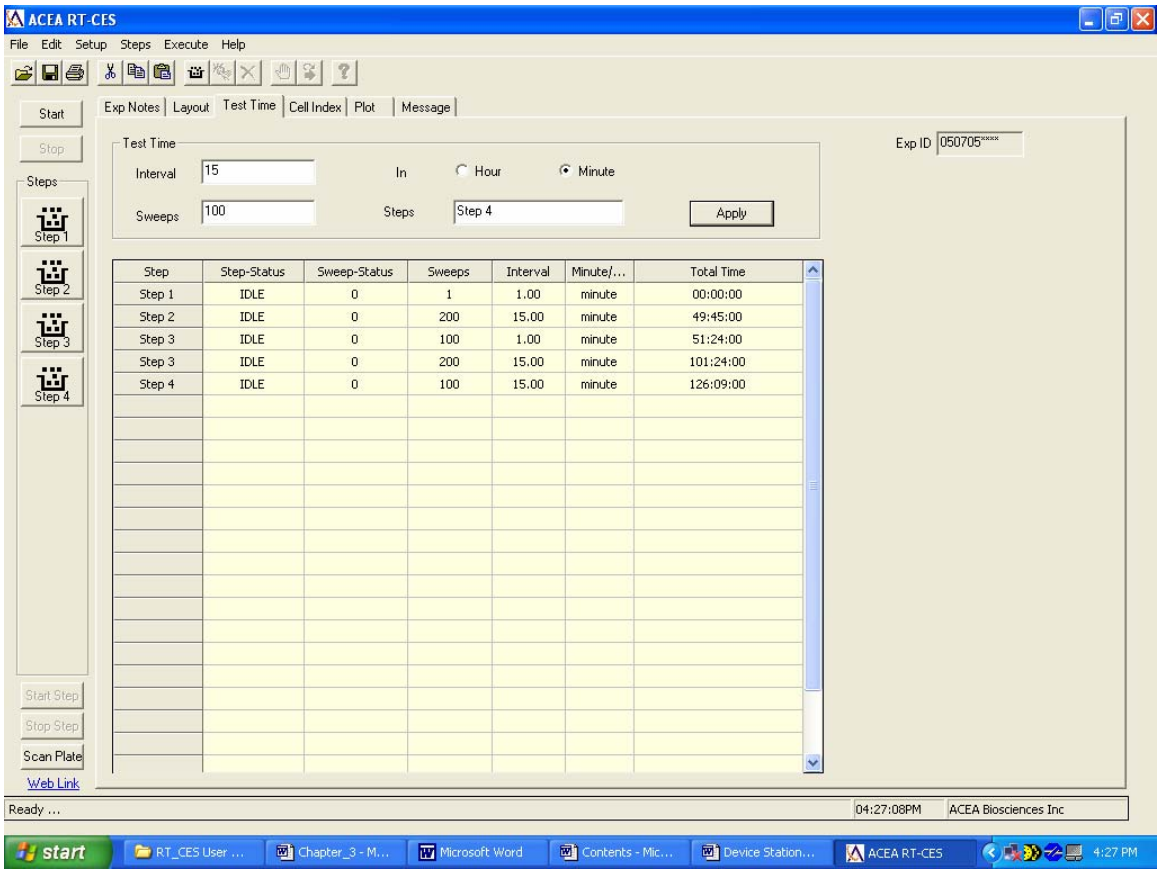
Step 2 is typically the first step after cell addition. Depending on the specific experiment, appropriate sweep numbers and time intervals can be set (for example, 200 sweeps, and 30 minutes). A large sweep number can be set and then manually stopped before completing all sweeps.

Step 3 typically comes after treatment (for example, compound addition) to the cells. Depending on the specific experiment, appropriate sweep numbers and time intervals can be set. For example, to measure rapid kinetics of cell responses to treatment, a time interval of one minute can be set. Within a step, sub-steps with different time intervals can be used. For example, step three can consist of 200 sweeps having one minute time intervals and another 200 sweeps having 15 minute time intervals.

To remove a step, highlight the step and click the Delete a Step icon , or go to the Steps menu and select Delete a Step.

See the **RT-CES SP / MP Software User's Manual** for more information regarding the Test Time page.

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Sample Test Time page

Start the experiment

1. Click **Start** to begin the experiment. This step measures the background impedance of cell culture media. The data is then used as reference impedance for calculating Cell Index values.
   During the experiment, the bottom of the program window displays "**testing column 1, 2, 3 … 12**", and the RT-CES Analyzer display reads "**Measurement in Progress….**".
   After completing the Step 1, RT-CES analyzer displays "**Next step to be started …**" and the bottom-left of the main program window displays "**Ready for Next Step. Please Click Next Step to start.**"

Remove the 16x E-Plate and add cells to the E-Plate wells

1. Remove the 16x E-Plate from the E-Plate Station by pulling the latch forward. The green light turns off.
2. Slide the pins of the 16x E-Plate out of the socket and remove the E-Plate from the incubator.
3. Add 100 µL of cell suspension into each well. The cell suspension has properly prepared cell concentrations for the cell titration experiment, and each well has proper cell numbers. Leave the E-Plate in the tissue culture hood for about 20 minutes at room temperature, so that the cells settle at the bottom of the well.

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Reinsert the 16X E-Plate and start step 2

1. Insert the 16X E-Plate to the same ZIF connector socket (20-holes each) used in step 1.
2. Lock the E-Plate to the connector by pushing the small lever located on the socket 90 degrees to the right. The green light turns on when it is connected properly.
3. Close the incubator door.
4. Start step 2 by clicking the Start Next Step icon . During the test, note that the bottom of the program window reads "**testing column 0, 1**", and the RT-CES Analyzer displays: "**Measurement in progress ….**"
   After completing each sweep, the RT-CES Analyzer displays "**Waiting for next sweep ….**" The bottom-left of the program window displays a count down to the next sweep start time (for example, "The time left is 0:05:36 for starting sweep 3, step 2").

Note:

1. *Make sure that all the twenty connector pins of the 16x E-Plate are straight and parallel to each other. If not, use finger or tweezers to straighten the "bent" connector pin. Failing in doing may lead to unreliable connection between the ZIF socket and the Resistor Board.*
2. *Make sure that all the twenty connector pins of the 16x E-Plate are clean and free from any dust or dirty particles. If not, wipe the connector pins with 70% alcohol and/or dust-off the connector pins with compressed air.*
3. *Make sure that all the twenty connector pins of the 16x E-Plate are properly aligned with the holes in the ZIF socket. If all the pins are straight and if all the pins are properly aligned with the ZIF socket, There is no need to push or force the 16x E-Plate into the ZIF socket. Forcing the connectors into the socket may damage the interior surface of the ZIF socket, leading to shortened life time for the ZIF socket.*
4. *Make sure that before inserting the 16x E-Plate, the small lever on the station is oriented parallel with the connector pins on the 16x E-Plates..*

Check and plot the cell index data

1. During the experiment and between sweeps, check the Cell Index or Plot page. In the Cell Index page, move to different test time points (the time is the time since the start of the experiment). Cell index values at the time point are shown in boxes.
   In the Plot page, plot the cell index for a well as a function of time, by highlighting the well, and clicking **ADD**. To add another well, repeat the process. For more information see **RT-CES SP/MP Software User Manual**.

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Running a quick RT-CIM experiment

Special Note: Please review the section of "Running a quick RT-CES experiment" before following instructions in this section. Many details in "Running a quick RT-CIM experiment" procedure are the same as those described in "Running a quick RT-CES experiment", and for simplicity, such details are omitted here.

Note: Before running the quick RT-CIM experiment, ensure that all the following items are checked:

1. Ensure that the RT-CIM System (including RT-CES analyzer and 16x RT-CIM station, computer with installed RT-CES SP software) is properly installed and that the two cable connections (one cable between the computer and RT-CES Analyzer and another cable between the RT-CES Analyzer and 16x RT-CIM Station) are properly linked.
2. During the check, if connections are redone, perform the resistor board verification again (see Resistor Board Verification -- for 16x E-Plate Station for more information).
   In addition, if the system has not been used for over a month, perform resistor board verification.
3. When the RT-CES analyzer is turned on, the front panel display reads:

   READY

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Note: If this is not what displays on the Analyzer, press the RESET button.

A simple cell migration experiment being run on a 16x RT-CIM device is used for illustration purpose. The procedure for this experiment includes:

- **Assembly of a migration device**
  1. Add media to bottom chamber
  2. Assembly of RT-CIM top chamber with bottom chamber
  3. Removal of excess fluid
  4. Add media to top chamber
- **Equilibrating the migration device in the incubator for 1 hour**
- **Starting the RT-CES SP software program *(during 1 hour incubation)***
- **Setting up ExpNote, Layout, and Test Time pages *(during 1 hour incubation)***
- **Positioning the migration device onto 16x RT-CIM station**
- **Starting the experiment and performing background measurement**
- **Removing the migration device and adding cells into the RT-CIM top chamber**
- **Re-positioning the migration device onto 16x RT-CIM station**
- **Start monitoring cell migration**
- **Checking and plotting the Cell Index data**

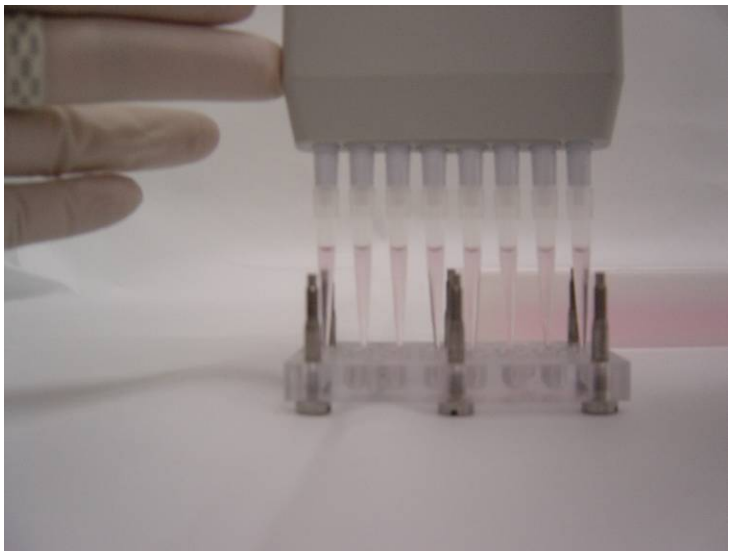
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Assembly of a migration device

1. Add media to bottom chamber

   a. Carefully fill the bottom chamber with ~ 151 uL of media using a (multi-well) pipetter. To ensure that bubbles are not trapped during the assembly of the device, **do not introduce any bubbles when adding the media into the well and make sure that a pronounced meniscus is formed on each well** (See following Figures).

   Note: With use, each user may have a preferred "volume setting" (e.g., 150 uL or 152 uL) on his/her pipette to fill the bottom chamber. There should be enough volume to form PRONOUNCED MENISCUS. But if there is too much media added to bottom chamber, then during "assembly of top chamber with bottom chamber" in Next Step, too much excess media may come out.



Media is added to the bottom chamber.

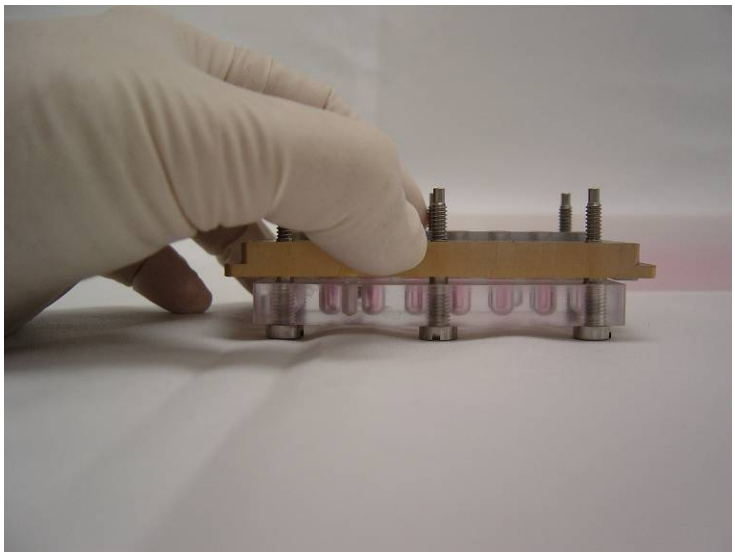


Pronounced meniscus is formed on each well.

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2. Assembly of RT-CIM top chamber with bottom chamber

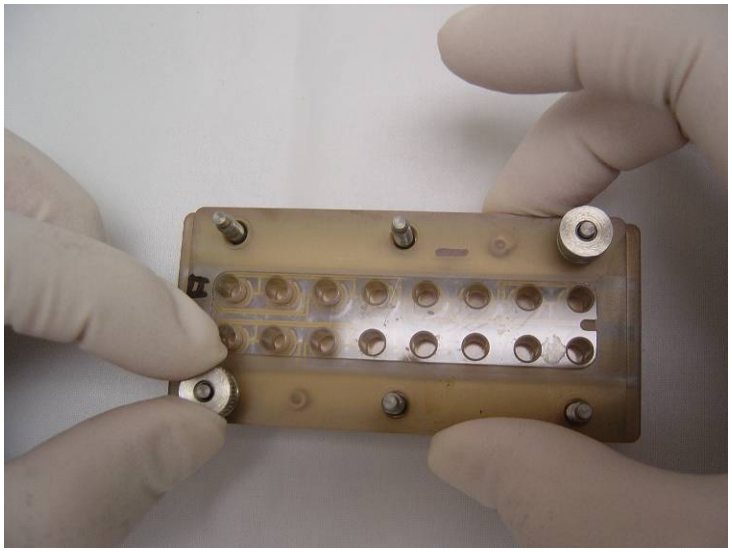
   a. Assemble the chambers by aligning the top chamber with the electrodes facing down on top of the bottom chamber, making sure the holes on the top chamber are in register with the screw posts on the bottom chamber. Lower the top chamber while keeping it as parallel to the bottom chamber as possible using the screw post as an alignment guide. This would prevent inadvertent trapping of bubbles while assembling the device. Once the top chamber is placed on top of the bottom chamber, keep an even downward-pressure on the two long-edges of the top chamber using your fingers to prevent it from moving. **Do not reposition the top chamber by lifting it off the bottom chamber or tipping one side of the top chamber as this will introduce bubbles**.



Keeping an even downward-pressure on the two long-edges of the top chamber.

   b. Secure the top chamber to the bottom chamber by alternately attaching the nuts provided with the device onto opposing screw posts. Place the first nut on the upper right screw post, tightening it down until a resistance is felt. Do not completely tighten the nut. Place the second nut on the screw post diagonally opposite the first screw post and tighten it again until a resistance is felt. **The goal is to keep an even pressure on the top chamber to the bottom chamber during assembly.** Repeat this procedure placing the third nut on the upper left, and followed by the fourth nut diagonally opposite the third screw post. Finish by placing the nuts on the center screw posts. Completely tighten the nuts going through the same sequence used when initially securing the nuts starting with the first nut in the upper right hand corner screw.

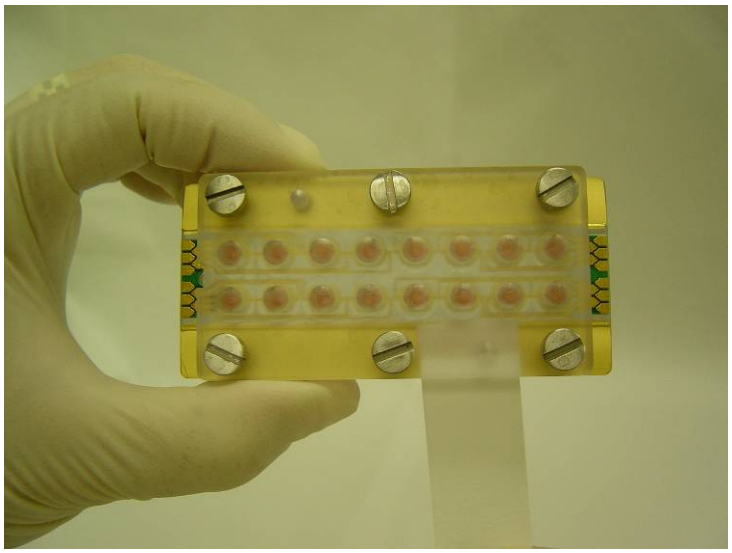
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Tightening the second nut (on the lower, left corner). **It is important to keep an even pressure on the top chamber to the bottom chamber during assembly.**

3. Removal of excess fluid

   a. Remove excess liquid spilling out of the silicon gasket. Turn the assembled device on its side and using ¾'' wide strips of Whatman paper, slide one end of the strip between the top and bottom chamber toward the silicon gasket. Wick as much excess fluid around the gasket ensuring that the area around the gasket is dry. **It is imperative that the area around the silicon gasket including the gold strips connecting to the PC board is dry as excess fluid can create a short in the circuit.**

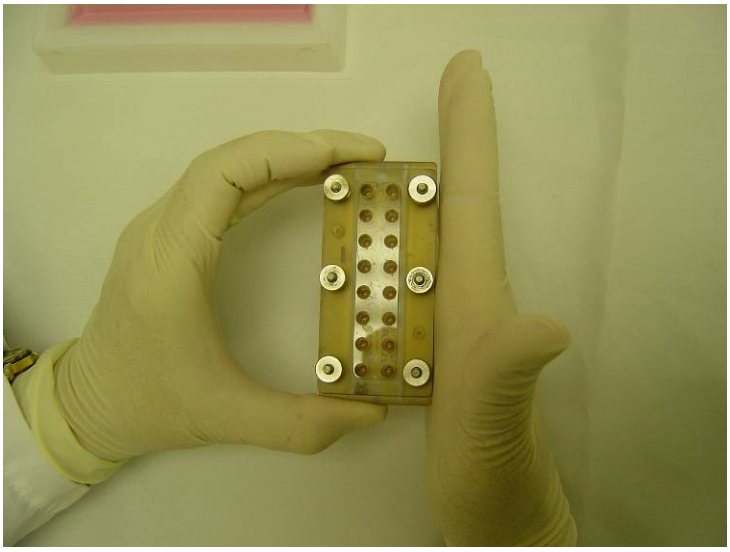


Use of Whatman paper to remove excess fluid coming out of the silicon gasket.

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4. Add media to top chamber

   a. Add 20 uL of media on the top chamber and tap either side of the device to evenly distribute the media over the membrane. To ensure that bubbles are not introduced into the top chamber, tilt the assembled device on its side while adding the media on the side wall of each well and as close to the membrane as possible. **Be careful not to puncture the membrane.**



Tap both long-sides of the device to evenly distribute the media over the membrane.

Equilibrating the migration device in the incubator for 1 hour

1. Placing the migration device into the 37°C/humidified incubator.

2. For optimum experimental result, the device should be left inside the incubator for about 1 hour to allow the surface of RT-CIM device to reach equilibrium with media applied at the bottom chamber.

Note:

1. It is OK to have the 16x RT-CIM device (i.e. the migration device) positioned inside a module of the 16x RT-CIM station during the 1 hour equilibration. Of course, the RT-CIM station is already inside the incubator. When placing the RT-CIM device onto the station, ensure that the RT-CIM device stay flat and NOT tilted. With the release-button pressed down, close the module cover. Let go the release button to lock down the RT-CIM module.

*2. During the 1 hr time of device being equilibrated in the incubator, start the RT-CES SP software program and set up ExpNote, Layout and Test Time pages. (**See Procedures Below**)*

*3. In addition, **cells for the cell invasion / migration experiment can be prepared during the 1 hr equilibrium time**.*

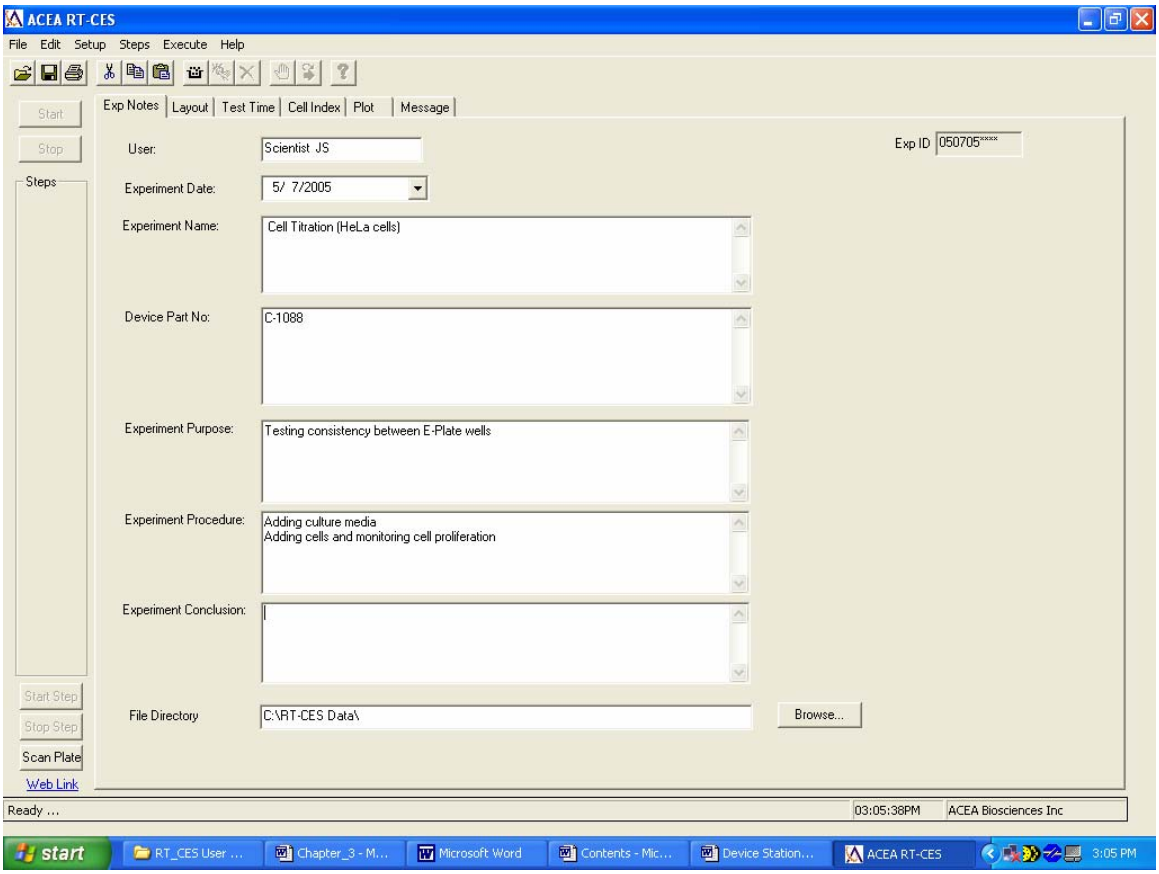
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Start the RT-CES SP program

1. Start the RT-CES SP software program by double clicking the ACEA RT-CES SP icon on the desktop (if the program is already open, close it, and then reopen it).

Set up the ExpNote, Layout, and Test Time pages

1. Set up the **ExpNote** page. This page records experiment-related information such as user, experiment date, experiment name, experimental purpose, experimental procedure, experimental conclusion, and the devices used in the experiment. Enter the information to record in the appropriate spaces. When the experiment starts, this information is automatically saved. You can also click **BROWSE** to select a File Directory to save the experiment files to. See **RT-CES SP / MP Software User's Manual** for more information about setting up the **EXPNote** page.



Sample Experiment Note Page

2. Set up the **Layout** page. This page determines the experiment layout and records experiment details about each well, including: name and the number of cells, name and concentration of compounds added into wells, etc.

Select the wells to be edited:

a. To select a single well, hover over the well to be selected, and left-click. The selected well is highlighted. The well ID of the selected well, is automatically filled into the Well IDs section. If, for example, the well D1 is selected, then the Well IDs section reads:" D1:D1".

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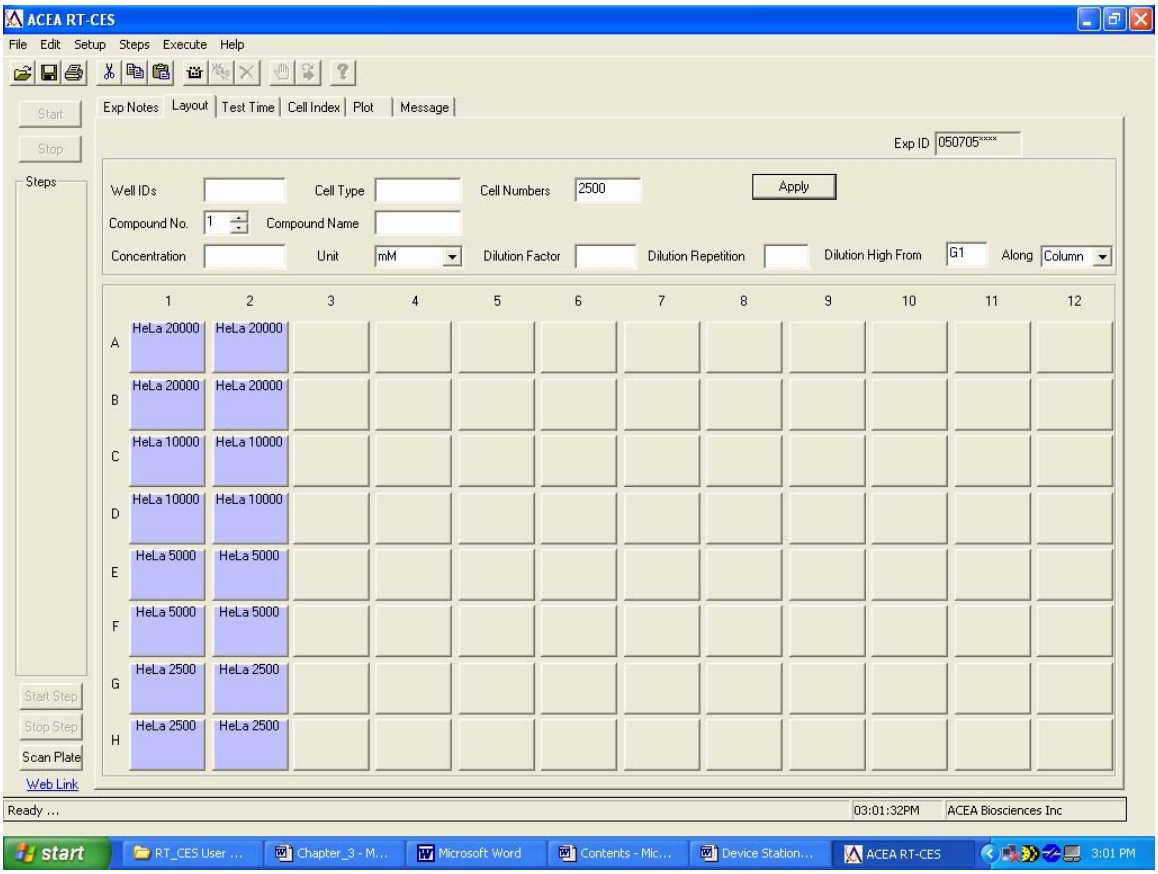
b. Enter the ID of the selected well directly into the Well IDs Box. If, for example, B2 is selected, enter B2:B2 into the Well IDs section. Or, if all wells between A1 and H2 are selected, type in A1:H2 into Well IDs section. The selected well is highlighted when the mouse moves from the Well IDs section to another section, such as Cell Type.

c. To select multiple wells, hover over the first well to be selected, left-click and hold the mouse. Drag the mouse across the other wells, to the last well to be selected. Release the mouse button. All selected wells are highlighted, the well's ID, for example A1:D2, is automatically filled into the Well IDs section, if all wells between A1 and D2 are selected. Note that the text in the Well IDs section has the format *First well ID: Last well ID*.

d. To select an entire row(s) or column(s), move the mouse to the row ID (e.g. A or E) or column ID (e.g. 1 or 3), and left-click. To select adjacent multiple-rows or multiple columns, left-click and drag across row IDs or column IDs.

Enter information into appropriate information edit boxes, and click **APPLY**. For example: Cell Type – MDA-231; Cell Number – 5000.

The other fields, including Compound No., Compound Name, Concentration, Unit, Dilution Factor, Dilution Repetition, Dilution High From, Along *Column or Row* is described in the **RT-CES/CIM SP/MP Software User's Manual**.



Sample Experiment Layout page

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3. Set up the **Test Time** page. This is used to program the experimental procedure. Generally, experiment can be divided into multiple steps, and each step consists of one or multiple sweeps.

Note: Each sweep is one measurement across all the selected wells. There is a pre-determined time interval between neighboring sweeps within a step. After completing a current sweep, the RT-CIM system automatically starts to count down the time interval and starts the next sweep.

After completing all of the sweeps within a step, the RT-CIM system does not move on to the next step until it is manually instructed to do so. There is always a manual intervention for the system to move from one step to the next one. Generally, each time the migration device is removed, there should be a separate step. Users can manually stop a current step before the system completes all pre-set sweeps. However, a current step cannot be manually stopped in the middle of a measurement sweep. Doing so may cause system instability and data loss.

Step 1 is typically used for background measurement, where no cells are present in the top chambers. It is typically set as One sweep, One minute. To add Step 1:

a. Move the mouse to the **Steps** column on the left side of the page.

b. Left click the Add a Step Icon , or go to the pull-down Steps menu and select Add a Step. Step 1 is added.

The Step Status is idle, and all other buttons are blank. Edit the Intervals and Sweeps section by entering the appropriate numbers. Select the Interval Units in hours or in minutes (or in seconds for RT-CES SP software with version No, 5.2 or higher). Click **Apply**, and the appropriate time intervals, with a unit and sweeps are added. The Sweep Status is automatically set to 0. Test Status displays as Test while the experiment is in progress. When the step is completed, Done displays. The Sweep Status section is updated and displays the number of sweeps that have been completed.

Note:

For RT-CES SP 5.2 or above, the sweep number and sweep interval for the Step 1 is pre-set by the software.

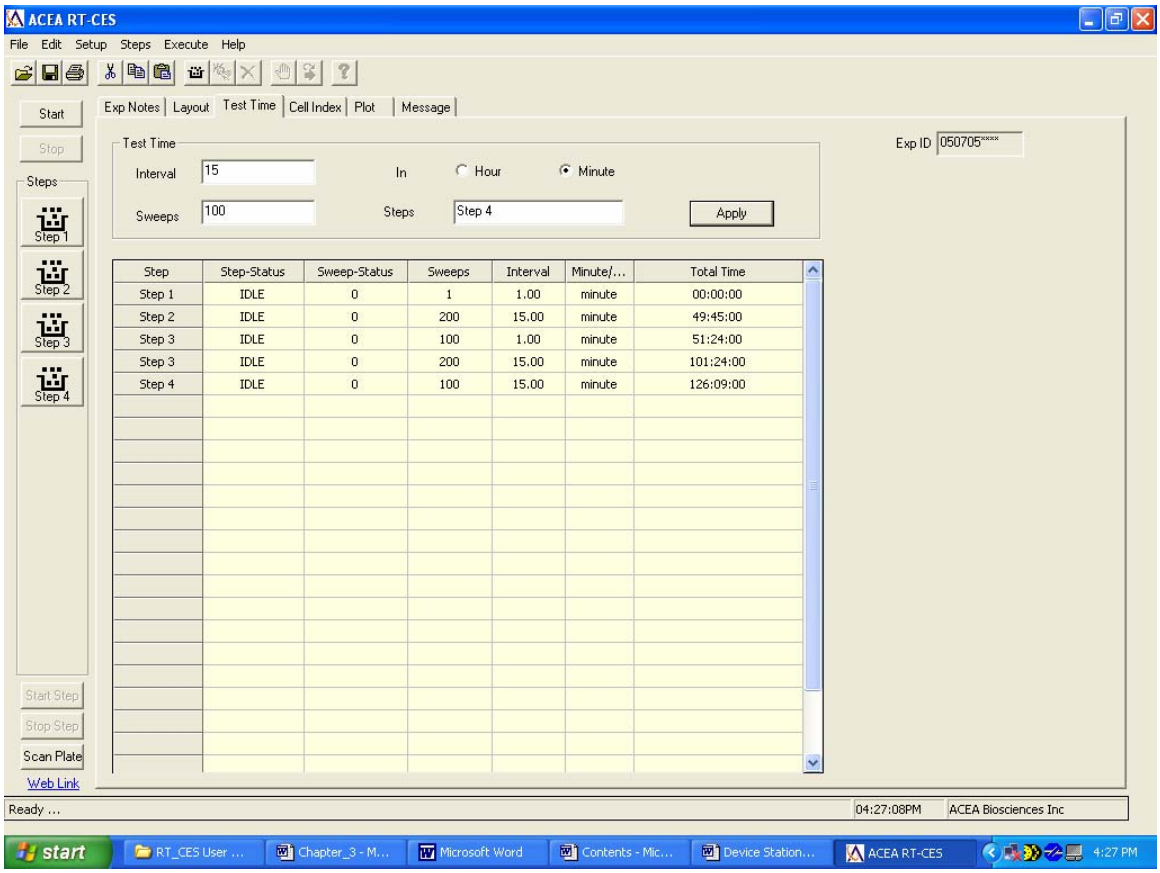
Step 2 is typically the first step after cell addition. Depending on the specific experiment, appropriate sweep numbers and time intervals can be set (for example, 60 sweeps, and 10 minutes per sweep). A large sweep number can be set and then manually stopped before completing all sweeps.

For Cell Migration or Cell Invasion assay, there may not be need for Step 3 or higher. However, depending on specific experiment and users' preference, one could add a Step 3 with appropriate Sweep numbers and Time intervals.

To remove a step, highlight the step and click the Delete a Step icon , or go to the Steps menu and select Delete a Step.

See the **RT-CES SP/MP Software User's Manual** for more information regarding the Test Time page.

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Sample Test Time page

Positioning the migration device onto 16x RT-CIM station

1. After 1 hour equilibration, place the 16x RT-CIM device into a module of the 16x RT-CIM station, which is inside the incubator. When inside the module, the RT-CIM device should be positioned flat and NOT tilted.
2. With the release-button pressed down, close the module cover. Let go the release button to lock down the RT-CIM module.

Note: The above step could be omitted if the CIM devices were positioned on the station during the 1 hour equilibration.

Starting the experiment and performing background measurement

1. After placing migration devices onto the CIM station, perform a background measurement of the device by Starting the experiment (Starting Step 1: 1 minute and 1 sweep). This step measures the background impedance of media in the bottom chamber. The data is then used as reference impedance for calculating Cell Index values.

During the experiment, the bottom of the program window displays "**testing column 1, 2, 3 … 12**", and the RT-CES Analyzer display reads "**Measurement in Progress….**". After completing the Step 1, RT-CES analyzer displays "**Next step to be started …**"

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and the bottom-left of the main program window displays "**Ready for Next Step. Please Click Next Step to start**."

Removing the migration device and adding cells into the RT-CIM top chambers

1. Press the release-button and lift the cover of the module on RT-CIM station.
2. Remove the migration device from the module. Remove the migration device from the incubator.
3. Add 100 uL of cell suspension samples on the top chamber. The cell suspension contains properly-prepared cell concentrations for the experiment. Thus, each top chamber has proper cell numbers. Leave the migration device in the tissue culture hood for about 20 minutes at room temperature, so that the cells settle at the chamber bottom.

   Note: Alternatively, prior to the addition of cell sample, remove residual volume of liquid in the top chamber. Make sure that the top chamber (i.e., the upper chamber) is not devoid of fluid for long periods of time by immediately adding cell sample solution.

Re-positioning the migration device onto 16x RT-CIM station

1. After cells being added to top chamber and the migration device being left at room temperature for ~ 20 mins, position the migration devices onto 16x RT-CIM station, which is inside the incubator. When inside the module, the migration device should be positioned flat and NOT tilted.
2. With the release-button pressed down, close the module cover. Let go the release button to lock down the RT-CIM module.
3. Close the incubator door.

Start monitoring cell migration

1. To start monitoring cell migration, continue experiment by starting Step 2 with a left click on the Start Next Step icon . During the test, note that the bottom of the program window reads "**testing column 0, 1**", and the RT-CES Analyzer displays: "**Measurement in progress ….**"
   After completing each sweep, the RT-CES Analyzer displays "**Waiting for next sweep ….**" The bottom-left of the program window displays a count down to the next sweep start time (for example, "The time left is 0:05:36 for starting sweep 3, step 2").

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Checking and plotting the cell index data

1. During the experiment and between sweeps, check the Cell Index page or Plot page.
2. In the Cell Index page, move to different test time points (the time is the time since the start of the experiment). Cell index values at the time point are shown in boxes.
3. In the Plot page, plot the cell index for a well as a function of time, by highlighting the well, and clicking **ADD**. To add another well, repeat the process.
   For more information see **RT-CES/CIM SP/MP Software User Manual**.

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Using RT-CES Analyzer

Initialization

When the RT-CES Analyzer is turned on, the Analyzer performs an automatic self-initialization. The front panel displays:

Ready

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The front panel buttons

Reset: Pressing the **Reset** button results in the front panel displaying:

Ready

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In addition, pressing Reset resets the analyzer to its default settings:

- Display Parameter: Default
- Monitor well on Row: A
- Monitor well on column: 1
- Test Frequency: 25,000 Hz
- Test Voltage: 20 mV

Mode: Pressing the **Mode** button changes the Analyzer mode to enable different parameters to be set.

If the Analyzer had the default settings initially, pressing the Mode button once displays the following on the front panel:

Display Parameter:

Default

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The parameter can be changed by pressing the **Parameter** button.

Pressing the **Mode** button again displays the following on the front panel**:**

Monitor well on row:

A

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The row number of the well to be monitored/displayed can be changed by pressing the **Parameter** button. There are eight row numbers: A, B, C, D, E, F, G, and H.

Pressing the **Mode** button again displays the following on the front panel:

Monitor well on column

1

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The column of the well to be monitored/displayed can be changed by pressing the **Parameter** button. There are eleven column numbers: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11.

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Pressing the **Mode** button again displays the following on the front panel:

Test frequency:

25,000 Hz

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Press the **Parameter** button to change the Analyzer's measuring frequency. There are three frequency parameters: 10,000 Hz, 25,000 Hz, and 50,000 Hz.

Pressing the **Mode** button again displays the following on the front panel:

Test voltage:

20 mV

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This is the only voltage setting for the Analyzer.

Parameter: Pressing the **Parameter** button (outside of the Mode settings) changes the parameter setting in the Analyzer. There are four parameters: Default, R(Resistance), Z(Impedance) and cell index of monitored well.

Pressing the Parameter button changes the display parameter between Default, R(Resistance), Z(Impedance), Cell index of monitored well.

The default setting enables the Analyzer to display messages after each sweep is completed, such as: "Waiting for next sweep …" or "Next step to be started …." If a different display parameter is selected, the Analyzer does not display messages. Instead, it displays results, such as:

Result for well A8:

Cell index = 1.888.

When a step is completed, the Analyzer displays step messages and results. For example:

Next step to be started …

R=128.58ohm

For Monitor well on row, pressing **Parameter** changes the row number of the well to be monitored/displayed between A, B, C, D, E, F, G, and H.

For Monitor well on column, pressing **Parameter** changes the column number of the well to be monitored/displayed between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

For Test frequency, pressing **Parameter** changes the frequency of the measurement by the Analyzer between 10,000 Hz, 25,000 Hz, and 50,000 Hz.

Test: Pressing the **Test button** enables the Analyzer to conduct one impedance measurement and display the measured results. The Analyzer displays:

Result for well A1:

R=58.888 ohm

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Using RT-CES/CIM SP and MP Software

There are seven pages in the RT-CES/CIM SP software. Three pages are used for running the experiments and three for monitoring experiments:

Experiment Note page: records key information about the experiment.

Experiment Layout page: designs the experiment.

Test Time Setting page: program the experiment steps and real-time data acquisitions.

Cell Index page: provides real-time data acquisition and monitoring.

Experiment Data Plot page: provides multiple data plot functions.

Data Analysis page: provides multiple data analysis functions.

Message Board page: monitors the quality of the sensor device and experiment process.

Refer to the **RT-CES/CIM SP/MP Software User's Manual** for detailed Information.

The RT-CES MP software was developed based on RT-CES SP software with additional functions to facilitate the measurement of up to six E-Plates. Main changes are:

A motor control and plate status page is added for controlling the status of six motor-modules and for displaying the measurement status for each plate;

For each E-plate, a 96-graph page is added for displaying the cell index curves for each individual well;

For each E-plate, there are six functional pages (the same pages as those in SP software, but not including Data Analysis page).

Important Tips for Using RT-CES/CIM System

ACEA Biosciences RT-CES/CIM system, 16x, 96x E-Plates and 16x RT-CIM devices are manufactured and tested according to rigorous quality standards. Each well in 16x/96x E-Plates and 16x RT-CIM top chambers is wet QC'd prior to packaging and shipment ensuring that every well in every E-Plate meets these quality standards.

In order to obtain optimal assay data quality and to avoid any problem with using RT-CES/CIM system, we request that the following procedures be observed when handling the RT-CES/CIM:

- Always perform background measurement of E-Plates (or migration devices) **with cell-free culture media**.

Without cell culture media, background measurement of E-Plates (or migration devices) cannot be performed. Similarly, background of E-Plates (or migration devices) cannot be measured properly with culture media *containing cells*.

- **Do not remove** E-Plates (or migration devices) from stations before completion of background measurement.

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If the E-Plates (or migration devices) are removed from station before completion of background measurement, then those wells whose background has not been measured would show an open-circuit for the background data. Experiments cannot be performed properly for these wells. A warning message is provided in RT-CES/CIM SP software with version number 5.2 or above.

For the same logic, please do not remove E-Plates (or migration devices) from station when the measurement or experiment is in progress. If E-Plates (or migration devices) are removed, the impedance for these E-Plates (or migration devices) would show open-circuit data.

- Always let the E-Plate devices (or migration devices) sit outside of the incubator for **at least 20 minutes** (or more, if total volume is over 150 micro-liter) after cell addition.

To get consistent data, cells need to settle down uniformly on the bottom surfaces of the E-Plate wells before the E-Plates are put into incubator. Otherwise, temperature-gradient resulted convection in the E-Plate wells would cause the cells to be distributed non-uniformly on the bottom surface of the wells – leading to large variation in experiment data. The above description is also true for migration devices.

- **Do not stop** an experiment or a particular step while the measurement is in progress.

Always wait until current measurement sweep finishes and then stop an experiment or a particular step. Otherwise, experiment data file may be corrupt and data cannot be retrieved.

- Each step must have **at least one sweep**.

For RT-CES/CIM SP software to run properly, at least one sweep is needed for any given step. Otherwise, data file format may not be correct. For RT-CES/CIM SP software with version number 5.2 or above, software will not continue for steps without sweep numbers.

- If 16x E-plates (or 16x migration devices) are removed from the station for whatever reason, they must be placed back on the station **in the same socket** as where they were initially located.

If 16x E-Plates (or 16x migration devices) are not placed back in the correct order, there would be serious problems with data collection since the data from different E-Plates (or migration devices) would be mixed up.

- **Do not re-use** the E-plates. **Do not re-use** the migration top chambers.

16x or 96x E-Plates are designed for one-time use only. Data quality cannot be guaranteed since surface properties of such re-used E-Plates would be altered from the tissue-culture-compatible properties of the un-used E-Plates. The above description is also true for the migration top chambers.

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- **Do not use some wells of an E-plate one day and use the remaining wells at another time.**

16x or 96x E-Plates are designed for one-time use only. If one uses some wells one day during an experiment, the other un-used wells on the same plate would be exposed to the incubator environment (5% CO2 with ~ 98% humidity) and their surface properties would be altered. Data quality cannot be guaranteed for these wells if they are used at another time.

- Seed **appropriate number** of the cells into the wells of E-plates.

Before running any assay on the RT-CES/CIM system, it is preferred to do a cell-titration experiment to test cell proliferation (or cell migration) for a range of cell numbers. This way would help to determine appropriate number of the cells used for the assay. If cell number is too high or too low, it would affect the data quality generated from RT-CES/CIM system.

- **Do mix** well the cell suspension during seeding cells into the wells.

Cells would settle down to the bottom of the cell-suspension tube. In order to have consistent cell number added to the E-Plates (or migration top chambers), it is essential to have cells uniformly (and randomly) suspended in the tube. Thus it is important to mix the cell suspension in the tube in-between adding cells to different wells.

- Make sure that all the 20-connector pins on 16x E-plates are c**lean and free of dust/dirty particles and free of any stains or spots, straight and parallel to each other**.

Clean and straight connector pins are essential for reliable contacts between E-Plates and 16x E-Plate station. If connector pins are dirty or if you are not sure, wipe the pins gently with 70% alcohol-soaked tissue (paper/cloth). If one or more connector pins are not straight, please gently bend the connector pins to make it straight.

- **No or little force** should be applied to push the 16x E-Plates to the socket.

When 16x E-Plates are inserted into sockets of the E-Plate station, no or little force should be applied to push the E-Plates to the socket. If excess force is needed, it means that one or more connector pins are NOT straight and may damage interior surface of the socket and also lead to un-reliable contact/connection between E-Plates and station. Un-straight pins can be gently bent to become straight.

- Make sure that all the 120-connector pads on the backside of the 96x E-Plates are **clean and free of dust/dirty particles.**

Clean connector pads (located on small, green PCB boards on the underside of the device plate) are essential for reliable contacts between E-Plates and 96x E-Plate station. If connector pads are dirty or if you are not sure, wipe the pads gently with 70% alcohol-soaked tissue (paper/cloth). Do not press too hard on such pads to avoid damaging the connection between the small PCB board to slides.

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- **Always do "Scan Plate"** when the 16x E-Plates are re-inserted back onto 16x E-Plate station after being removed.

After performing "Scan Plate", please check Message Page for scan results to see whether you have some wells have connection problems, If yes, remove the 16x E-Plate and re-insert it on the E-Plate station. Before you re-insert the E-Plate, please check to see whether the 16x E-Plate connector pins are clean. Or, you may just go ahead to clean connector pins (16x E-Plates) before re-inserting E-Plates for another "scan plate" test.

Importantly, for re-inserting 16x E-Plates into the station sockets, sometime you can tilt the 16x E-Plate slightly (connector pins lower than rest of the 16x E-Plates) so that the E-Plates are inserted into the socket with a small angle (less than 10 degrees). This may provide better contacts since the connector pins now contact different points inside the sockets. If you do so, the E-Plates along its lengthy direction may not be exactly-horizontal, which will not affect any of your measurements.

If second "Scan Plate" shows same problem as the 1st "Scan Plate", you may continue experiments with this plate, or you may want to try again with a new plate (assuming that the cells have not been added). You should report such incident to ACEA Biosciences representative and provide the Serial Number of the 16x Device Plate send appropriate data files (see Trouble Shooting section for details).

- **Always do "Scan Plate"** when the 96x E-Plates are re-positioned back onto E-Plate station after being removed.

After performing "Scan Plate", please check Message Page for scan results to see whether you have some wells have connection problems, If yes, two options should be tried.

1. With the 96x E-Plate on the station and in the "down" position (SP, the front handle in lower position and locked; MP the module in the DOWN position), you may want to ***press the plate down all the way twice on the 1st-column end and twice on the 12nd-column end***. This action can sometimes remove the dust between the Pogo pin and the pad to allow better contact. You can then do a scan plate which may show that the "connection-problem" well is OK now.
2. The second option is to remove the 96x E-Plate and re-position it on the E-Plate station. Before you re-position the E-Plate, please check to see whether the 96x E-Plate connector pads are clean. Or, you may just go ahead to clean connector pads (96x E-Plates) before re-positioning E-Plates for another "scan plate" test.

If second "Scan Plate" shows same problem as the 1st "Scan Plate", you may want to continue the experiment with this plate, or you may want to try again with a new plate (assuming that the cells have not been added). You should report such incident to ACEA Biosciences representative and provide the serial number of the Device Plate and the appropriate data files (see Trouble Shooting section for details).

- Please **turn off** RT-CES Analyzer if the system is not used for several days or longer period of time.

  Just like any electronic equipment, if the RT-CES system is not used, please turn it off. It will prolong the life of the equipment and save electricity.

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- **Always** clean bottom-chambers of RT-CIM devices **immediately after** the cell migration/invasion experiments are complete. Follow the cleaning protocol with care.

a. After each use, without delay, clean the bottom chambers by soaking in a solution of abut 0.1% SDS/0.1 M NaOH for 1 hour. Shake the container every once a while (ten minutes) to ensure that cell particulates are dislodged from the bottom well surface. Or alternatively, pipetting up and down the solutions for each well several times once a while (10 minutes).

b. Thoroughly rinse the bottom chamber with running water. Make sure that all the residual salt and SDS solutions are completely rinsed off.

c. Soak components in distilled or de-ionized water for 15 minutes.

d. Thoroughly rinse the bottom device with running water. Perform a final wash with Milli-Q water (or water filtered at least at 18 MOhm).

e. Remove residual water from each well in the bottom plate with a disposable pipette tip on a suction line, and let chamber components air dry at room temperature under a protective paper towel, or blow dry with **Clean** compressed gas, e.g. nitrogen.

f. To sterilize, spray the bottom well with 75% solution of ethanol in Milli-Q water. Remove excess fluid by flicking the device. Let stand in the hood under UV to sterilize and air dry for 30-45 minutes.

- **Periodically** clean bottom-chambers of RT-CIM devices **using enzyme active detergent** to remove proteins or contaminants accumulated on the bottom chamber walls.

Proteins and other contaminants sometimes accumulate as deposits on the bottom chamber walls. This can happen quickly depending on the media being used in the experiment. Accumulation or deposit is immediate and severe if protein solutions are ever allowed to dry in the wells. These deposits may affect the migration or invasion of the cells that are assayed with the devices. In a worst situation, the deposits may be cytotoxic.

Periodic soaking of all the bottom chamber components in about 1% Terg-a-Zyme® (an enzyme active detergent available from most major laboratory suppliers or contact Alconox, Inc.) for about 1 hour at 50°C (or overnight at room temperature) usually removes these residue deposits. Follow with thorough rinsing under running distilled or de-ionized water and soaking in same de-ionized water overnight.

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Maintenance and Troubleshooting

Maintenance

There are no internal serviceable parts in RT-CES analyzer, E-Plate (16x or 96x) and 16x RT-CIM station.

Cleaning

Important: If the system will not be used for a long period of time (a month or more), pack the RT-CES analyzer and E-Plate (16x or/and 96x and/or MultE-Plate) station in the original plastic bags inside the shipment boxes.

Warning: The RT-CES analyzer weighs approximately 15 lbs (7.8 kg) and should be lifted with care. Two people should lift the instrument together, taking proper precautions to avoid injury.

When you are ready to use the instrument again, please refer to the Installation section for details on unpacking and installation.

The surface of the RT-CES Analyzer and E-Plate (16x or/and 96x) station should be cleaned and wiped with a towel that has been *lightly* soaked in alcohol. Ensure this is done for E-Plate Stations that will go inside an incubator.

Fuse Replacement

	The RT-CES Analyzer utilizes 2-pole fusing. Before attempting to remove/replace fuses, ensure that the On/Off switch is in the Off (0) position and that the power cord has been removed from the AC receptacle. Take care to use only Type FSF 5 x 20 fast acting fuses with a 250V, 2A rating.

1. Using a small, flat screwdriver, depress the snaps on both sides of the fuse drawer.

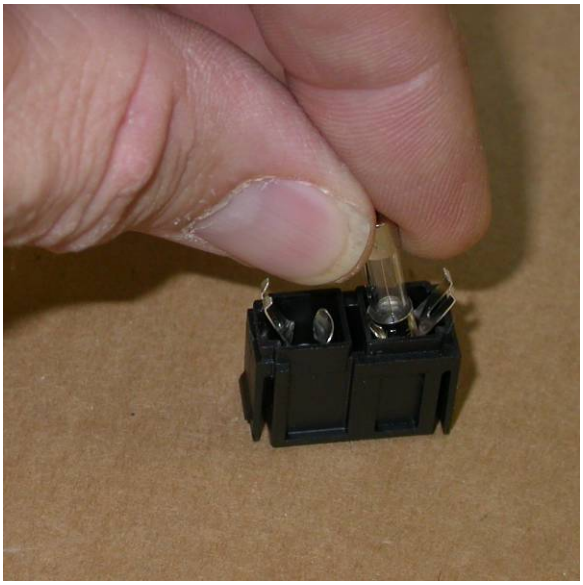


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2. Pull the fuse drawer out of the AC Receptacle. The fuses should remain connected to the drawer.



3. Remove the old fuses from the drawer.
4. Insert new fuses as shown.



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5. Press the fuse drawer, along with the fuses, into the AC Receptacle. Ensure that both ends of the drawer snap completely into place.



Resistor Board Verification

If the system has not been used for a long period of time (a month or more), perform resistor board verification to confirm that all connections are secure and that all components are working properly.

Please refer to the Installation section for Resistor Board Verification for both 16x station (RT-CES and RT-CIM) and 96x stations (RT-CES).

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Troubleshooting

I started an experiment and there was an "Incorrect ACK Command, Please Check the System" message.

The computer failed the communication with RT-CES analyzer. Please check that the cable linking the computer to the analyzer is securely connected.

If this happened during the initial Scan Plate test, do the following:

1. Close the RT-CES/CIM SP (or RT-CES MP) software.
2. Turn off the analyzer.
3. Disconnect the cable from the Analyzer and from the computer.
4. Reconnect the cable.
5. Turn on the Analyzer and press the Reset button on the front of the Analyzer.
6. Restart the RT-CES/CIM software.
7. Restart the experiment.

If this happened after you pressed the Start button in the software, do the following:

1. Turn off the analyzer.
2. Disconnect the cable from the Analyzer and from the computer.
3. Reconnect the cable.
4. Turn on the Analyzer and press the Reset button on the front of the Analyzer.
5. Restart the experiment.

If this happened in the middle of an experiment, do the following:

1. Close the RT-CES/CIM SP (or RT-CES MP) software.
2. Turn off the Analyzer.
3. Disconnect the cable from the Analyzer and from the computer.
4. Reconnect the cable.
5. Turn on the Analyzer and press the Reset button on the front of the Analyzer.
6. Go to the appropriate file folder and use the RT-CES/CIM SP (or RT-CES MP) software to open the experiment that was stopped.
7. Restart the experiment.

How do I reinitialize the RT-CES System after a power failure?

1. When the power is restored, restart your computer system. Do not open the RT-CES/CIM SP/MP software at this time.
2. Turn off the Analyzer. Wait for 2-3 seconds, and then turn it back on.
3. Open the RT-CES/CIM software and locate the data file folder that was generated before and during the power failure. Open the file folder.
4. Click the Resume button in the top left corner of the software. The experiment starts where it left off when power was interrupted.

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The cell index signal is significantly lower than usual. Why?

You need take the E-Plate devices out of the incubator and check the cell status (cell number, cell distribution and morphology) under microscope.

1. Maybe the initial seeding cell number was not correct.
2. Maybe you forgot the step of "leaving the E-Plate device (or migration devices) at room temperature for about 20 minutes" after the cells were seeded. If that step was not done, then cell distribution would not be uniform over the well surface. The cell index signals would be low, especially for the corner wells.
3. Maybe there is some type of contamination and cells are dying off.
4. Maybe the cells were harvested from an over-confluent flask. Our experience is that if the cells in the flask reached a confluence over 80%, then the cells may not be in the LOG phase and the cell status may not be at its best. Such cells when seeded onto an E-Plate (or migration devices) may give a low cell index signal.
5. Maybe there is something wrong with the incubator. One likely symptom with incubator problem is that the cell index is lower on edge wells than the center wells or cell index goes to nearly zero quicker on the edges than the center wells. The problems with incubators may include CO_2 level, temperature control, humidity control and certain chemicals released from the incubator. We have encountered several instances where the incubator itself was presenting to be "toxic" or affecting cell growth. Sometimes a simple but thorough cleaning of incubator interior may be sufficient to solve the problems. Alternatively, simply running experiment in another incubator may solve all the problems.

Please make sure that if you take the E-Plate devices out of the incubator, the instrument is not performing a measurement. If the instrument is "waiting for next sweep", make sure that you have enough time to check the cells under microscope. If you do not have time, you may have to stop the current step and then take the device out of the incubator for checking. And then Resume the experiment after putting the devices back in.

The cell index signal is decreasing with time, but there was no treatment. Why?

Take the E-Plate devices out of the incubator and check the cell status (cell number, cell distribution and morphology) under a microscope. Check for the following:

1. Contamination resulting in cells dying.
2. Culture media that is too old to support the cell growth, because the experiment is too long.
3. Culture media quantity is insufficient to support cell growth, due to too much evaporation.
4. Maybe there is something wrong with the incubator. One likely symptom with incubator problem is that the cell index is lower on edge wells than the center wells or cell index goes to nearly zero quicker on the edges than the center wells. The problems with incubators may include CO_2 level, temperature control, humidity control and certain chemicals released from the incubator. We have encountered several instances where the incubator itself was presenting to be "toxic" or affecting cell growth. Sometimes a simple but thorough cleaning of incubator interior may be sufficient to solve the problems. Alternatively, simply running experiment in another incubator may solve all the problems.

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If you take E-Plate devices out of the incubator, ensure that the instrument is not performing a measurement. If the instrument displays “Waiting for next sweep,” make sure there is enough time to check the cells under a microscope. If you do not have sufficient time, stop the current step, and remove the device from the incubator. Then, replace the device and resume the experiment.

The cell index signal is significantly higher than usual. Why?

Remove the E-Plate devices from the incubator and check the cell status (cell number, cell distribution and morphology) under a microscope. It is possible that the initial seeding number was high.

If you take E-Plate devices out of the incubator, ensure that the instrument is not performing a measurement. If the instrument displays “Waiting for next sweep,” make sure there is enough time to check the cells under a microscope. If you do not have sufficient time, stop the current step, and remove the device from the incubator. Then, replace the device and resume the experiment.

I did background measurement. Software indicated many wells (more than 2 wells per 16x device) might have background problems. What should I do ?

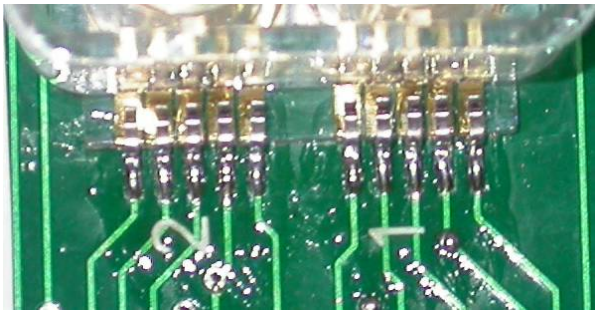
Please perform “scan plate” . Check Message Page for scan results to see whether you have many wells showing connection problems. Alternatively, you can check plate scanning data file located in current experimental directory (file name: PlateScanning) to see whether any well shows open-circuit (i.e., the resistance values are abnormally high (for example, more than 300 ohm) or negative.

If the message shows that there are many cells having connection problems or if the plate-scanning data file shows many wells having open-circuits, then we may have problems with E-Plate devices or E-Plate station or both. Before we conclude such issue, please perform an additional “scan plate” after removing E-Plates in question from E-plate Station and then re-inserting/re-positioning back to E-Plate Station. Check the plate-scan results again, as described above, to see whether connection-problem wells or open-circuit wells are about the same as before. If problems did not show up in the 2nd scan-plate, experiments can be continued. The initial issue might have something to do with the insertion or positioning of E-Plates into/onto E-Plate Stations.

If connection-problem wells or open-circuit wells are about the same as before, we would conclude that we have problems with E-Plate devices or E-Plate station or both.

First, based on the figures below, you can check to see whether there is an alignment problem between glass slides and metal clips on PCB board. If so, such devices are defect, contact ACEA Biosciences Sales or Technical Support and send to ACEA these E-Plates together with appropriate data files (see question below). Such problems may occur because of in-proper handling in shipping/receiving. ACEA Biosciences will continue to work with shipping companies to avoid in-proper handling.

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Correct

Alignment between slide and PCB



In-correct

Mis-alignment between slide and PCB

Second, if there is no mis-alignment between glass slides and metal clips, check whether all connector pins on 16x E-Plate devices are straight and parallel to each other and/or whether all pins on 16x E-Plates or all connector pads on 96x E-Plates are clean and free of any dust/dirty particles. If not, please gently bend connector pins to make all the pins straight or clean the connector pins on 16x E-Plates or the connector pads on 96x E-Plates with 70%-alcohol-soaked tissue. After such cleaning, please re-insert or re-position E-Plates for another Scan-Plate test. If number of open-circuit wells is the same as before, then please report such problems to ACEA Biosciences Sales team or Technical Support and send appropriate data files (see question below). You may decide to continue the experiments for E-Plates or wells in E-Plates without background problems.

I need to switch my E-Plate stations from 16x (or 96x) to 96x (or 16x). How do I do that?

Please refer to the Installation section for the details.

I have some concerns with measured cell index curves or I discovered some problems with E-Plates (or migration devices) or E-Plate Stations (or RT-CIM Stations). What should I do ?

Please contact ACEA Biosciences Sales team or Technical Support and send appropriate data files (see question below).

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I am contacting ACEA Biosciences Sales team or Technical Support. What data files should be sent?

For any experiments you have doubt or concerns about either E-Plates or systems, please ALWAYS SEND the following data files: 3 INDEX files, 6 PAGE file, 1 Calibration.txt file, and six T0 file and six T1 files. Do not include any confidential information. If any information is confidential, please remove information discuss with ACEA Sales Team or Technical Support to learn how to NOT include such confidential information.

Examples of such files are as follows.

(1) Three Index Files.

1108051838Index1.idx

1108051838Index2.idx

1108051838Index3.idx

(2) Six PGE Files

1108051838ENotes.pge

1108051838ETimes.pge

1108051838Freqcy.pge

1108051838Layout.pge

1108051838Mesage.pge

1108051838TTimes.pge

(3) One .TXT file

Calibration.txt

(4) Six T0 files

0106061257OrgT0Plate0Freq10000.txt

0106061257OrgT0Plate0Freq25000.txt

0106061257OrgT0Plate0Freq50000.txt

0106061257T0Plate0Freq10000.txt

0106061257T0Plate0Freq25000.txt

0106061257T0Plate0Freq50000.txt

(5) Six T1 files

0106061257OrgT1Plate0Freq10000.txt

0106061257OrgT1Plate0Freq25000.txt

0106061257OrgT1Plate0Freq50000.txt

0106061257T1Plate0Freq10000.txt

0106061257T1Plate0Freq25000.txt

0106061257T1Plate0Freq50000.txt

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