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TopCount Topics

TCA-016

ViewPlates™: Methodology for Counting Isotopic and Luminescent Labels for Use in Adherent Cell Assays

Abstract

Adherent cell assays for reporter genes, cell adhesion, chemotaxis, cell viability, cytotoxicity, and receptor binding may be performed and counted in 96-well ViewPlates™ or CulturPlates™ (Packard Instrument Company, Meriden, Connecticut) on the TopCount Microplate Scintillation and Luminescence Counter. ViewPlates and CulturPlates are tissue culture-treated to provide optimal cell growth and adhesion. ViewPlates have optically transparent bottoms to enable microscopic viewing of cells, and opaque walls to minimize well-to-well crosstalk. ViewPlates are available with white or black walls, and may be used for either isotopic or luminescent labels. CulturPlates are white, opaque microplates for use when microscopic viewing is not required. This paper describes optimal methods for counting ViewPlates on TopCount and demonstrates the growth of an adherent cell line on ViewPlates.

Introduction

When clear polystyrene microplates are used for liquid scintillation or luminescent counting, optical crosstalk is a major problem. Attempts to measure these samples in clear polystyrene microplates by implementing software correction schemes for crosstalk have failed due to large statistical errors when measuring low energy isotopes such as ^{3}H and ^{125}I. In order to physically eliminate optical crosstalk, Packard has developed ViewPlates and CulturPlates.

ViewPlates are designed with a clear bottom to allow microscopic viewing of cells, an essential step in confirming cell viability. CulturPlates have an opaque bottom and are intended for use in situations in which microscopic viewing is not required. The combination of ViewPlates and CulturPlates with TopCount enables the investigator to perform adherent cell assays, such as reporter gene, cell adhesion, chemotaxis, cell viability, cytotoxicity, or receptor binding assays, within the same plate without transferring cells for counting. By performing all steps from cell culture to quantification in one plate, replicate variation is lower, sample identification is ensured, throughput is increased, and costs are reduced.

ViewPlates are molded from polystyrene in the standard 96-well format, then tissue culture-treated. The plates are individually packaged with lids and sterilized. The combination of opaque side walls and clear well bottoms enables microscopic viewing of cells and minimizes the effect of optical crosstalk during counting. ViewPlates are available with white or black side walls for applications involving either isotopic or luminescent labels. CulturPlates are white (opaque) polystyrene microplates which are manufactured and tissue culture-treated by Nunc® for Packard Instrument Company.

This paper will focus on how to use ViewPlates for optimal counting performance. Data are presented on counting efficiency and crosstalk for isotopes commonly used in adherent cell assays (^{3}H, ^{14}C, ^{32}P, ^{45}Ca, ^{125}I). Also included are crosstalk data from the measurement of a glow chemiluminescent chemistry.

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Methods

For all isotopic experiments, white ViewPlates were used with MicroScint-20 (Packard Instrument Company). MicroScint-40 may also be used. The following radioisotopes were purchased from Amersham® (Arlington Heights, Illinois): ^{125}I (Cat. #IMS.30, sodium iodide); ^{32}P (Cat. #PBS.13A, orthophosphate); ^{45}Ca (Cat. # CES.3, calcium chloride); ^{3}H (Cat. # TRK.565, thymidine); and ^{14}C (Cat. #CFA.532, thymidine).

Isotopes were diluted in MicroScint-20 to yield the desired activity level, then used at 100 µl/well. Control wells were filled with 100 µl of MicroScint-20 without radioactivity. ViewPlates were sealed with TopSeal™-S (Packard Instrument Company), then white or black backing tape was applied to the bottom of the plate. After a one minute count delay, the plates were counted at 19 °C for one minute/well on the TopCount.

The backing tapes are pre-cut, opaque adhesive sheets which are included with ViewPlates for attachment following microscopic examination of the cells. White backing tape maximizes counting efficiency for ^{3}H and black tape is recommended for crosstalk reduction of all other isotopes.

Crosstalk was calculated by dividing the average counts from eight surrounding control wells by the counts in the center well containing the radioisotope. Counting efficiency was based on the DPM data from 100 µl of the isotope stock solution counted on a Tri-Carb® 2500TR liquid scintillation counter in 10 ml of Ultima Gold™ (both manufactured by Packard Instrument Company).

Chemiluminescence was evaluated using a lucigenin substrate triggered with an alkaline solution in a final volume of 200 µl/well. Control wells contained lucigenin substrate plus a non-activating neutral solution. Both black and white ViewPlates were evaluated. Plates were sealed using TopSeal-A (Packard Instrument Company), and then black backing tape was applied to the bottom of the plate. TopSeal-A is an adhesive cover seal which does not require a heat sealing device. Heat may adversely affect chemiluminescent reactions. After a three minute count delay, the plates were counted at 25 °C on TopCount in single photon mode for 0.02 minute/well.

Chinese Hamster Ovary (CHO) cells were grown to confluency in white ViewPlates and a commercially available plastic tissue culture dish. CHO cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal calf serum and 1% penicillin-streptomycin and incubated for 48 hours at 37 °C with 5% CO_2. Cells were photographed using a Leitz® Orthomat E™ camera mounted onto a Leitz Diavert™ microscope using a 12.5 x 40 magnification.

Isotope	ViewPlate	Tape	Channels	Efficiency*	Crosstalk
H-3	white	white	2-256	23.7%	0.69%
C-14	white	black	5-256	62.8%	0.05%
Ca-45	white	black	8-256	70.3%	0.09%
P-32	white	black	5-256	72.5%	0.21%
I-125	white	black	2.9-256	23.3%	0.20%
Luminescence	white	black	n/a	n/a	0.24%
Luminescence	black	black	n/a	n/a	0.003%

*Actual counting efficiencies for labels used in adherent cell assays may be lower due to quenching and self-absorption effects of the cell material in the sample.

Table 1.

Summary of optimal counting parameters for ViewPlates on TopCount. The column titled "ViewPlate" refers to the color of the ViewPlate walls, white or black. The column titled "Tape" refers to the color of backing tape, white or black, which was applied to the bottom of the ViewPlate. n/a stands for not applicable.

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Results

Radioisotope Counting

The data collected using isotopic and luminescent labels are summarized in Table 1. White backing tape increases counting efficiency by reflecting light. Black backing tape reduces optical crosstalk by absorbing light and thereby prevents light reflection into adjacent wells. Another strategy which was used to reduce crosstalk with high (>100 keV) energy beta emitters was to raise the lower counting region limit. This decreased crosstalk with only a minimal loss of counting efficiency. The data presented in Figure 1 represent the actual CPM without any background subtraction or manipulation of the data with crosstalk correction software.

^{3}H: Tritium was counted using white backing tape with counting region limits (in channels) set at 2.0-256. Since tritium is a low energy beta emitter, white backing tape was used to optimize counting efficiency by providing a reflective surface. Under these conditions, the counting efficiency for tritium was 23.7%, and crosstalk was 0.69%.

^{14}C: Carbon was counted using black backing tape, and the counting region limits were set at 5.0-256. Black backing tape is recommended for all beta emitters with an energy which is higher than that of tritium. Under these conditions, the counting efficiency for carbon was 62.8%, and crosstalk was 0.05%.

^{45}Ca: Calcium was counted using black backing tape, and the counting region limits were set at 8.0-256. The counting efficiency was 70.3%, and crosstalk was 0.09%.

^{32}P: Phosphorous was counted using black backing tape, and the counting region limits were set at 5.0-256. The counting efficiency was 72.5%, and crosstalk was 0.21%.

^{125}I: Iodine was counted using black backing tape, and the counting region limits were set at 2.9-256. The counting efficiency was 23.3%, and crosstalk was 0.20%.

Luminescence Counting

Chemiluminescent and bioluminescent chemistries can be measured in white or black ViewPlates. Black backing tape should be used with

Carbon-14				
12	11	10	3	4
3	18	109	14	10
13	142	126689	123	10
6	13	113	17	12
6	12	6	11	6

Calcium-45				
6	3	3	6	3
9	21	215	17	9
5	219	150546	434	5
3	19	123	27	1
4	3	5	5	5

Phosphorous-32				
2	3	3	1	3
2	121	296	103	6
1	298	93188	280	0
0	103	311	91	4
6	1	1	4	2

Iodine-125				
54	55	67	58	70
64	150	392	156	106
69	310	91866	368	131
62	160	358	190	113
47	59	50	81	92

Figure 1.

Radioisotopes were diluted in MicroScint-20 and pipetted into the center well of white ViewPlates. Surrounding wells contained cocktail only. The data are presented in CPM with no background subtraction. The samples were counted on TopCount according to the optimal parameters for each isotope as outlined in Table 1.

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Luminescence										
White ViewPlate						Black ViewPlate				
225	252	269	225	252		93	77	82	66	71
269	1756	2631	831	280		77	93	82	71	77
324	9073	2455562	9706	341		66	93	424076	82	88
269	5962	12223	7245	363		71	82	93	82	82
275	330	352	341	313		82	88	82	88	77

Figure 2.

A luminescent substrate was added to white and black ViewPlates and counted on TopCount in single photon mode as described in the Methods section. The data are presented in CPS with no background subtraction. Black backing tape was used for both white and black ViewPlates.

both types of plates. The same volume and molar concentration of lucigenin substrate was measured in white and black ViewPlates. Crosstalk was 100 fold lower in the black plates. Counting efficiency was 5.8 times greater in the white plates due to the greater reflectivity of the white walls. Choice of plate color will depend o n the strength of the luminescent signal. The CPS data for luminescence counting are presented in Figure 2.

Cell Growth and Attachment

In order to demonstrate cell growth, Chinese Hamster Ovary cells were grown in white ViewPlates, Figure 3. CHO cells were also grown in standard tissue culture dishes as a control standard for normal cell growth, Figure 4. The CHO cells grew well on both surfaces. After 48 hours, CHO cells had reached confluency on the white ViewPlates, whereas the CHO cells on the tissue culture dishes had not yet reached confluency, due to the larger surface area.

Discussion

The ability to perform cellular assays, such as reporter gene, cell adhesion, chemotaxis, and receptor binding, in a single plate all the way through from cell plating to quantification, is now possible using ViewPlates in combination with TopCount. ViewPlates are ideal for cell-based assays because their clear well bottoms enable microscopic examination of cell viability, and their opaque side walls and patented design minimize optical crosstalk.

Isotopic labels commonly used in cellular assays, such as ^{3}H, ^{14}C, ^{45}Ca, ^{32}P, and ^{125}I, can be measured using white ViewPlates with black backing tape, except for tritium which should be measured with white backing tape. Luminescent labels may be measured in white or black ViewPlates with black backing tape. Table 1 provides a summary of the optimal counting parameters for use on TopCount.

Since polystyrene is hydrophobic in its untreated state, ViewPlates are treated by corona discharge. This procedure inserts carboxyl groups into the polystyrene backbone, resulting in a hydrophilic surface with a net negative charge to promote cell growth and adhesion.[1] The ViewPlates are then packaged individually with lids and sterilized by gamma irradiation.

For situations in which microscopic viewing of cells is not required, CulturPlates are ideal. CulturPlates are white (opaque) polystyrene 96-well microplates which are manufactured and tissue culture-treated by Nunc for Packard Instrument Company.

References

1. Ramsey, W.S., *et al.*, Surface treatments and cell attachment. *In Vitro* 20, 802-808 (1984).

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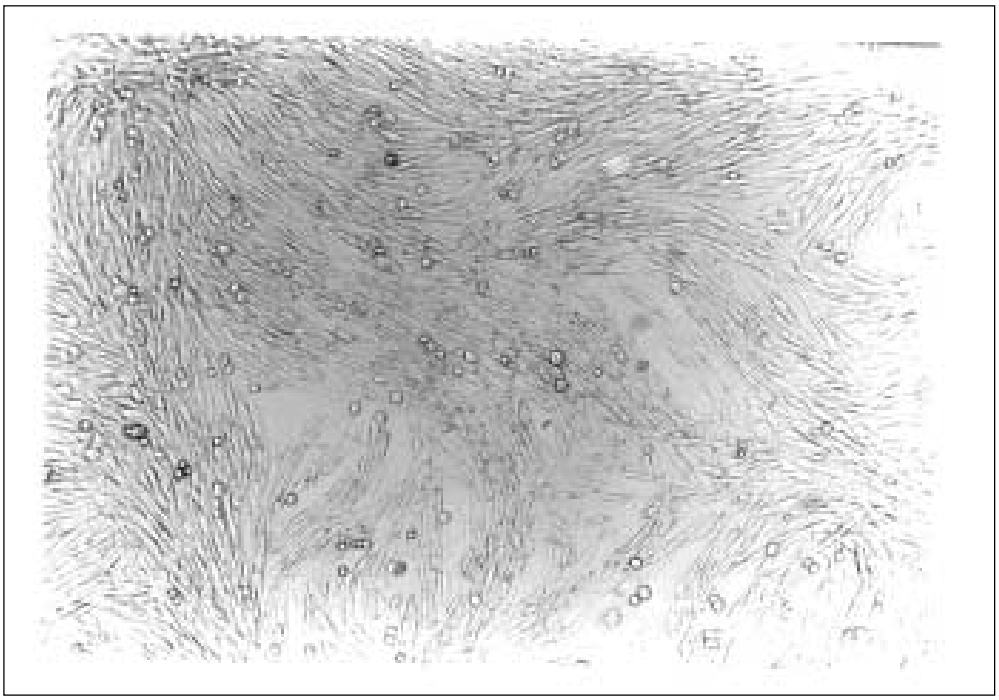


Figure 3.
CHO cells were grown in white ViewPlates as detailed in the Methods section.
This photograph was taken after 48 hours of culture.

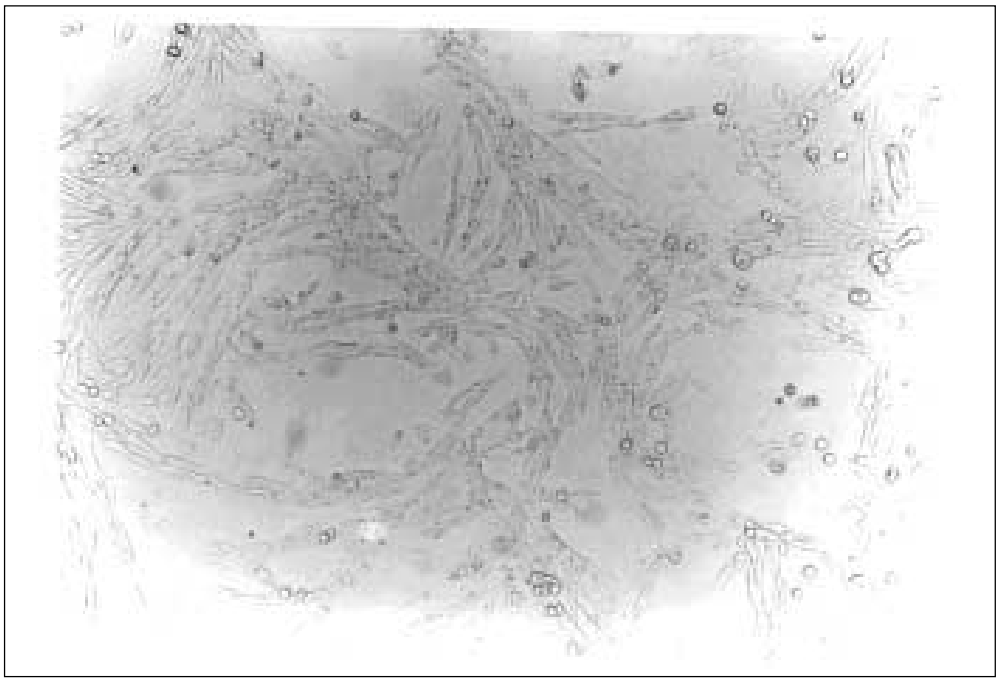


Figure 4.
CHO cells were grown in Falcon tissue culture dishes as detailed in the Methods section.
This photograph was taken after 48 hours of culture.

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