QUADRA 3® Application Note

Automation of Filtration Protocols

The application flexibility of the Quadra 3 liquid handling workstation is enhanced with multiple application specific accessories. Not least of which is the operator's ability to interchange pipetting heads to optimize specific results. Precision and accuracy are provided in the same workstation from 450µL down to Nanoliter volumes. (See Quadra 3 pipetting heads)

Using application specific accessories utilizes the Quadra 3-walkaway automation capability. Typical applications are those involving the use of vacuum driven filtration. Tomtec has developed an automated vacuum box to reside on the Quadra 3 shuttle. A small robotic hand automatically transfers microplates and filter plates from the Quadra 3 stackers in and out of the vacuum box.

Not only can a filter plate be placed on the vacuum box for evacuation but another conventional or deep well plate, may be placed inside as a capture plate. This capture plate may be a filter plate for those applications requiring dual filtration, such as the Millipore Montage kit. If capture of the eluant is not required, it will automatically go to waste via a small vacuum regulated vacuum trap.

By stacking multiple filter plates and capture plates within the Quadra 3 stackers, complex filtration protocols can be run in a walkaway, automated fashion. One typical application, using Pall's Mustang plates, required 5 different eluants of 1 ml each, of which 3 captures of 1 ml each were in to 3 different deep well plates. The Quadra 3 ran that application flawlessly in 18 minutes unattended and repeated 2 more times without operation intervention.

The ability of the robotic hand to move microplates and filter plates from the stackers to and from the vacuum box provides unlimited flexibility in automating protocols. The Quadra's pipetting dispense rate is matched to the vacuum driven flow rate through the filter plate to eliminate any overflow. A vacuum regulator on the vacuum trap controls the applied vacuum. Therefore, the applied vacuum can be matched to the filter plate requirement. This flexibility fits the most complex filtration protocols, providing multiple plate runs in an unattended mode of operation.

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The Quadra 3 program is maintained in a memory and can be recalled at the touch of a button. Each Quadra 3 program generates its own checksum. If the checksum matches the original program checksum, the Quadra 3 will run exactly the same program with an error rate of less than $1x2^{32}$ *(1 in 4 trillion).*

Pneumatics provides high force in a fast acting, compact package for reliable mechanical operations, time after time. The Quadra 3, uses it's own self-contained pneumatic system. For vacuum filtration, the user simply provides a raw vacuum source and electrical power. The Quadra 3 takes it from there with reliability in an unattended operation. All of these features in a compact 30" of benchtop space.

Montage Plasmid Kit for the Tomtec Quadra 3 with Vacuum Box and Arm

In genomic research, the first order of business is to extract DNA from some source. First and foremost, the target DNA must be taken from the species to be used. The target DNA is then chopped into smaller fragments that can be easily used. The smaller fragments are "pasted" into another known span of DNA. This creates a circular plasmid. The importantance of plasmids is two fold; One, they can be easily inserted into common E. coli bacteria and two, they are replicated inside the bacteria as it multiplies quickly in a nutrient broth.

These plasmids represent a basic tool in genomics research. Due to its ease of use and well-understood characteristics, the plasmid is used to "store" DNA fragments in a bacterium, which can easily be frozen for long term storage. To use the plasmid in a working phase, it must first be purified from the bacterium.

Several essential elements come into play in order to purify this plasmid DNA. Plasmid DNA is kept inside the bacterium in multiple copies. The plasmids are considered free floating in the cytosol which is the main compartment of the cell. Within the cell there exists another DNA type, which is the genomic DNA of the growing cell. This type of DNA is the coding material for the bacteria and is essential to direct most processes within the cell. There is only one copy of the genomic DNA per bacterium. It is important to note that the bacterium cannot survive without the genomic DNA, but can survive without plasmid DNA.

Typically, the cells containing the plasmid are grown up in nutrient broth in a deep well microplate. After a suitable incubation period for growth and multiplication, the deep well plates are centrifuged to locate the cellular mass in a pellet at the bottom of the wells. The supernate is emptied. This is normally done manually by flipping the plate upside down over a suitable receptacle.

The pelleted, plasmid deep well plates are placed in the Quadra 3 stackers. The Quadra transfers resuspension liquid into the deep well plates to resuspend and lyse

the cells. Typically, this is a solution of sodium hydroxide; NaOH. After a short lysing time, an acidic solution, typically HCL, is added to neutralize the sodium hydroxide to stop the lysing action.

The Quadra tip capacity of 450µL minimizes the number of liquid transfers for this lysing and neutralizing action. Normally, the contact time between the addition of the NaOH and the neutralization with HCL is short, i.e. 2 to 5 minutes. The Quadra easily accomplishes this by processing the plates in pairs.

The first plate infeeds from the stacker to the shuttle (station #1) and receives NaOH. The 2nd plate infeeds from the stacker to the shuttle (station #2). NaOH is added to the second plate. HCL is aspirated from a shuttle reservoir (station 5) and added to plate #1 and then #2 . If the sequential processing time between plates is too short for the protocol, then a" timed pause" may be added to the program. The "timed pause" allows the time lines on each plate may be matched to the protocol. This sequence is repeated for plates 3 & 4, 5 & 6, etc. with the Millipore Montage Kit.

Following the neutralization step, the two filter plates (clearing plate and plasmid plate) infeed from the right rear stacker where they have been sequentially stacked. The plasmid plate infeeds first. The robotic hand places the plasmid plate within the vacuum box as a capture plate. The clearing plate is next placed on top of the vacuum box. The neutralized lysate is transferred from the cell plate and added to the clearing plate. The eluent is captured in the plasmid plate within the vacuum box. The vacuum regulator on the vacuum trap is set to provide the appropriate vacuum flow rate for the protocol. The dispense rate of the Quadra tips may be matched to the vacuum rate to prevent overflow.

At the completion of this step the program turns the vacuum off. The hand transfers the used clearing plate to the right front stacker. The plasmid plate is retrieved from within the vacuum box and is placed now on top of the vacuum box. The required amount of wash solution is added to the plasmid plate using the Quadra tips. The vacuum draws this through the filter membrane and it is collected in the vacuum trap.

The desired, washed, plasmid result resides on top of the filter in the plasmid plate. The vacuum is shut off. Resuspension liquid is added, mixed, and aspirated off the filter surface. This is then transferred to the final collection plate that infeeds from the right rear stacker. It is part of the 3 plate set, i.e. plasmid plate, clearing plate, and collection plate.

Following the completion of the processing of the first cell plate in the set, it is repeated on the second plate. The approximate time to process each cell plate is about 5 minutes plus the actual vacuum filtration time. The vacuum filtration time

is a function of the available vacuum, types of cells being used, cell density, and other similar factors.

The above describes the use of Millipore's Montage Kit for plasmid purification. Additional specific details are shown on Millipore's website www.millipore.com.

The protocol for plasmid purification using Pall's product is slightly different. Pall does not use a two-plate process. With their single plate protocol, plasmid purification is achieved by washing a single plate with various salt concentrations to achieve the desired end results.

Regardless of the specific protocol being used, the flexibility of the Quadra 3 workstation can achieve the desired processing results of multiple plate sets in an unattended mode of operation.

Montage PCR Kit for the Tomtec Quadra 3 with Vacuum Box and Arm

PCR is a basic tool for molecular biologists. The overall concept is to create numerous copies of fragments of DNA. This is accomplished through the addition of several components and cycling the temperature. In a simple PCR reaction, certain components must be added: a master copy of DNA, nucleotides or DNA building blocks, primers or starting fragments of DNA and a polymerase, which is an enzyme used to link the nucleotides onto the primers which all use the master copy as a template. There are of course other materials contained in the reaction such as salt buffers. The entire mixture is then heated to various temperatures for the reaction to occur. It is as this point that the PCR fragments (copies) must be purified from the mixture.

Again, an isolation / purification procedure is important for downstream processing of the fragments. Why? Because many of the various salts and polymerase can interfere with subsequent chemical reactions necessary to produce final data. Thus, many researchers use purification kits such as the Montage PCR kit from Millipore. This kit contains a filter plate, which captures the DNA on the plate allowing the salts, polymerase, nucleotides and primers to pass through. The DNA copies of interest (PCR product) are then removed off of the top surface of the filter plate.

The researcher can automate this process utilizing the Quadra 3 platform. The filtration process takes place in an automated vacuum box, which accepts the filter plates and allows the filtrate to pass through into a vacuum trap. The liquid handling capabilities of the Quadra 3 make this an ideal automation solution. Without considering the vacuum time (which can be highly variable depending on the starting material), the Quadra 3 can process a full PCR plate in less than 3 minutes.

Montage Zip Kit for the Tomtec Quadra 3 with Vacuum Box and Arm

In proteomics research, many researchers need to purify their proteins before downstream applications such as Mass Spec. The entire sample may be contaminated with other elements that need to be isolated from the material of interest. The purification agent used in the Zip Tip kit is standard C18, which is normally used for SPE on larger volumes. However, the amount of C18 used in each of the ZipTip plate wells is geared towards the smaller scale requirements for detection in mass spectrometers.

Researchers may separate proteins from a complex mixture such as a cell lysate or a matrix such as an acrylamide gel. Once the proteins are partially separated, the protein spot may be excised for further work. The protein spots have been stained with an agent, which can create downstream problems. Thus, further purification is necessary. This is where the ZipTip plate utilizes a solid phase separation technique to isolate the protein of interest. These partially purified proteins can then be analyzed through a mass spectrometer.

Although the moniker of solid phase extraction is correct, a researcher may assume that the filtration product is akin to a miniature chromatography column for very small samples. It is this process of “easy to use” purification, that Tomtec has automated on the Quadra 3 for use with the Montage ZipTip kit. The liquid handling platform (Quadra 3) utilizes an automated vacuum filtration box with an associated robotic hand. The time to process one set of 96 purifications is less than six minutes, without the vacuum time. Please note that the vacuum time can be variable depending upon the starting material.