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ProductInformation

UltraClear™ Sequencing Reaction Clean-Up Plates

UC9601, UC9604

Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The UltraClear™ Sequencing Reaction Clean-Up Plates offer a rapid and simple method for the clean up of DNA sequencing reactions in a 96-well format. The plates use ultrafiltration membranes to separate low molecular weight contaminants, such as unincorporated dye terminators, dNTPs, and residual salts from the sequencing reaction products.

Following thermocycling, the sequencing reactions are diluted with Sequencing Solution. This mixture is then filtered through the UltraClear plate by centrifugation.

The sequencing reaction products are retained on the surface of the ultrafiltration membrane while the lower molecular weight contaminants pass through the membrane and are collected as waste. The purified sequencing products are then resuspended in Sequencing Solution and are ready for injection onto capillary-based DNA analyzers.

Components	Product Code	UC9601 1 x 96	UC9604 4 x 96
UltraClear Sequencing Reaction Clean-Up Plates	U 4633	1 plate	4 plates
Sequencing Solution (0.3 mM EDTA)	S 1944	25 ml	100 ml

Equipment and Reagents Required But Not Provided

- Centrifuge capable of holding multi-well plates and attaining at least 1000 x g.
- 96-well receiver plate with a minimum capacity of 200 µl per well. (Product No. M 8185).
- Injection plate for intended capillary sequencer.
- Plate shaker for resuspending sequencing reactions. (Optional)
- Automated liquid handler for resuspending sequencing reactions. (Optional)

Precautions and Disclaimer

The Sigma Ultraclear Sequencing Reaction Clean-up Plates are for R&D use only, not for drug, household or other uses. Please consult the material safety data sheet (MSDS) for information regarding hazards and safe handling practices.

Preparation Instructions

After the purification steps are performed, the purified product on the membrane will need to be resuspended in Sequencing Solution by agitation. In addition to manual agitation, this may be performed by using one of the following methods:

- **Automated liquid handler pipetting:** If an automated liquid handler will be used, the protocol should incorporate pipetting the sequencing solution up and down in each well for 20 cycles. The pipette tips need to be within 0.5 cm of the membrane.

- **Plate shaking:**
  A platform shaker or vortexer fitted with a plate adapter may be used. The device used will need to be calibrated prior to running the procedure. Refer to Appendix 1 for calibration instructions.

Suggested devices and settings:

-Scientific Industries Vortex Genie-2 equipped with a micro titer plate adapter. The vortexer should be set between shake 3 and vortex 3.

-Thermolyne AROS 160 Adjustable Reciprocating Orbital Shaker set at 240 rpm.

Storage/Stability

Store the kit at room temperature (18-25 °C).

Procedure

This procedure is optimized for the efficient clean up of sequencing reactions containing 2 µl or less of BigDye® Terminator v3.1 in a total reaction volume of 10 µl or less. If an alternative reaction scale or dye terminator is being used, the procedure may need to be modified. See the troubleshooting guide for suggestions to enhance performance.

1. Dilute Sequencing Reactions
   Following thermocycling, dilute reactions to 60 µl with Sequencing Solution. For example, to a 10 µl reaction, add 50 µl of Sequencing Solution.

2. Load UltraClear Plate
   Transfer the diluted sequencing reactions onto the center of the membranes in the UltraClear plate wells.

3. Prepare Plate Assembly
   Place the UltraClear plate containing the diluted sequencing reactions securely onto a receiver plate and place this stacked plate assembly into the centrifuge. This receiver plate will capture waste during the filtering step.

Note: The receiver plate needs to fit snuggly to the bottom of the filter plate and withstand the centrifugation forces used in Step 4.

4. Centrifuge the Plate Assembly
   Centrifuge the plate assembly according to the Centrifugation Options table below or until all liquid has completely passed through all wells. Note that centrifugation settings are in units of force (x *g)* not units of speed (rpm). Refer to Appendix 2 for conversion of centrifugal force to speed.

Centrifugation Options

Centrifugal Force	**Time**
1000 x *g*	30 minutes
1500 x *g*	25 minutes
2500 x *g*	20 minutes
3500 x *g* (recommended)	15 minutes

Note: For optimal results it is important that all liquid flows completely through the membrane. Additional centrifugation time may be necessary to completely remove all residual liquid from the membrane prior to proceeding to resuspend step. Longer centrifugation times are generally necessary when the top of the plate is sealed with tape during centrifugation.

5. Add Sequencing Solution
   Remove the stacked plate assembly from the centrifuge and discard the receiver plate.
   Add 40 µl of Sequencing Solution to the center of the membranes in the UltraClear plate wells.

6. Resuspend the Purified Sequencing Products
   Resuspend the purified sequencing products by one of the following methods.

   - Recommended method: Shake the plate for 5 minutes on a plate shaker that has been previously calibrated as described in Appendix 1.

   - Manually shake the plate horizontally in rapid circular motions on the lab bench top for 30 seconds then allow the plate to sit at rest for 5 minutes at room temperature.

   - Allow the plate to sit at rest for 20 minutes at room temperature.

   - Pipette up and down for 20 cycles using automated liquid handler.

Note: Manual pipetting, even with a multichannel pipettor, is not a reliable method of resuspending the purified sequencing products and can result in poor resuspension and high well-to-well variability. Therefore, manual up and down cycling with a pipettor is strongly discouraged.

Transfer to the Injection Plate

Transfer the resuspended sequencing products to an appropriate injection plate as defined by instrument manufacturer. Due to the small amount of retention, 35 µl or less should be removed by aspiration from each well. This will ensure consistent volumes of each purified sequencing product. The purified products in Sequencing Solution are now ready for immediate injection onto a capillary-based DNA analyzer or for storage at –20 °C.

Reference

1. BigDye Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems, Foster City, CA.

Troubleshooting Guide

Problem	Cause	Solution
Poor sequence data	Improper reaction conditions	Ensure a control sequencing reaction is performed during each thermocycling procedure and optimize reaction conditions if necessary. Refer to the BigDye Terminator v3.1 Cycle Sequencing Kit protocol available from Applied Biosystems.
	Insufficient liquid flow through the membrane	Centrifugation settings must be of sufficient speed and time to provide enough force for all liquid to completely pass through the membrane during Step 4 of the Procedure.
	Manual pipetting for resuspension.	Use plate shaker, liquid handler, or other option listed in Step 6 of the Procedure for resuspending purified reaction products.
Excess unincorporated dye terminator remaining	Concentration of dye terminators in reaction too high	Procedure is optimized for 2 µl or less of ABI BigDye Terminator v3.1. If possible, use 2 µl or less. Refer to the BigDye Terminator v3.1 Cycle Sequencing Kit protocol available from Applied Biosystems.
	Inadequate consumption of dye terminators	Refer to the BigDye Terminator v3.1 Cycle Sequencing Kit protocol available from Applied Biosystems for optimizing reaction conditions.
	Insufficient removal of excess dye terminators	Perform a 60-µl wash step after the completion of filter Step 4. This wash is performed by adding 60 µl Sequencing Solution to all wells of the UltraClear plate that contain sequencing reactions. The UltraClear plate is then placed onto the receiver plate. Centrifuge the stacked plate assembly for the same time and force used in Step 4 of the Procedure. Proceed to Step 5 of Procedure to resuspend purified sequence products.
Low signal strength	Low reaction yield or improper injection conditions	Resuspend the purified reactions using Molecular Biology grade water instead of the Sequencing Solution during Steps 5 and 6 of Procedure.
Cross over contamination between wells	Shaking too fast	Calibrate shaker. Do not allow liquid to flow over the top of the wells into adjoining wells.

Related Products	Product Codes
10x CE Buffer	B4930
SeqSaver Premix Dilution Buffer	S3938
Water for Molecular Biology	W4502
Thermowell® PCR plate	P3606

Appendix 1 - Plate Shaker Calibration

Perform the following optimization when using a plate shaker to resuspend the purified sequencing reaction products.

1. Place a standard flat-bottom 96-well plate or a previously used UltraClear plate loaded with 100 µl of water onto the shaker. A dye solution may be used for better visualization of the liquid.

2. Beginning at a lowest setting, start shaking the plate.

3. Increase the shaking speed until the liquid begins to spill out of the wells.

4. Reduce shaking speed until the liquid does not spill out. Record or mark this setting for use in Step 6 of the procedure.

Appendix 2 – Conversion of Centrifugal Force to Centrifuge Speed

All centrifugation settings in this protocol are given in units of gravity (*g).* An accurate determination of centrifugal force is important for optimal performance of the UltraClear Sequencing Reaction Cleanup Plates. Conversion tables are usually provided in the owner's manual of the centrifuge. Alternatively, force may be determined from speed and the radius of the centrifuge rotor according to the equation:

$$RPM = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

Where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*; *r* = radius of the rotor in cm; *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

For example: An IEC PR-7000M model centrifuge equipped with a 966 model rotor and DoubleDeep Microplate carriers has a radius, r, equal to 21.8 cm. To achieve an RCF of 3500 x g, the centrifuge speed should be set to 3790 RPM.

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