SEQueaky Kleen H_20™ Dye Terminator Removal Kit

Instruction Manual

Catalog Number

732-6500

For technical service
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On the Web at http://www.discover.bio-rad.com

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Section 1. Introduction

The SEQueaky Kleen H_2O™ dye terminator removal system utilizes a gel filtration matrix with optimal particle size, pore size, and bed volume. This system achieves maximally efficient capture and segregation of unincorporated Big Dye* terminators, salts, and other low molecular weight species, with a maximum yield of cycle sequencing products. The gel filtration matrix completely removes unincorporated dye terminators, provides high signal intensity during both gel- and capillary-based fluorescent sequencing, and eliminates the need for excessive spin times or g-force. Slurried in nanopure water, with no additional buffering agents or salts, each well of the purification plate is prepacked with fully hydrated matrix and ready to use.

Section 2. Kit Components

Bio-Rad gel filtration plate, 2

Collection plate with lid, 4

Instruction manual

*Big Dye is a trademark of Applera

Section 3. Instructions for Use

1. Remove sealing tape from the top and bottom of the SEQueaky Kleen H_2O plate and cover with the lid of a collection plate.
2. Place the SEQueaky Kleen H_2O plate with lid on top of a collection plate and transfer the assembly into a rotor with the appropriate plate stage. The brake should be set at maximum.
3. Centrifuge for 2 min at 750 x g to purge residual water from the matrix bed. Begin timing as soon as centrifugation begins. Disregard the short time required for the rotor to stop rotating.

   Note: For 50 µl samples, centrifuge for 5 min at 750 x g to purge.
4. Remove the plate assembly from the centrifuge. Discard both the lid and collection plate. Transfer the SEQueaky Kleen H_2O plate onto a clean collection plate.
5. Pipet the cycle sequencing reaction samples (≤50 µl) onto the center of the matrix bed in each well. Avoid penetration of the matrix bed with the pipet tip or discharging the sample between the matrix bed and the wall of the well.

   Note: For 10 µl samples, we recommend adding 10 µl deionized water to the sample prior to pipetting it onto the matrix bed. This will generally result in increased product recovery and increased signal intensity during electrophoretic analysis.
6. Transfer the SEQueaky Kleen H_2O plate assembly with lid back into the centrifuge. Centrifuge for 2 minutes at 750 x g to recover the purified sample. Begin timing as soon as centrifugation begins.

Section 4. Quick Guide

Place a SEQueaky Kleen H_2O filter plate with lid on top of collection plate

Place into tabletop centrifuge with microplate carrier rotor

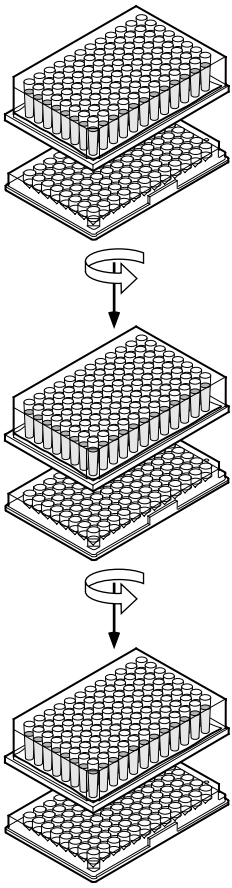
Centrifuge 2 min at 750 x g to remove residual buffer

Change collection plate

Load sequencing reactions (≤ 50 µl)

Centrifuge 2 min at 750 x g to remove Big Dye* terminators, low molecular weight species, and salts

Purified DNA of sequencing reactions is recovered in collection plate



Section 5. Optimal Filtrate Range

The following table indicates the range of optimal filtrate volumes recovered when purifying different samples or deficient filtrate volumes for which an increased spin time or g-force is recommended.

Sample volume	Expected filtrate volume	Comments
10 µl	12–15 µl	Increase spin if filtrate is <10 µl
20 µl	18–22 µl	Increase spin if filtrate is <15 µl
50 µl	30–35 µl	Increase spin if filtrate is <25 µl

Section 6. Troubleshooting Guide

Problem	Possible Cause	Possible Solutions
Low filtrate volume	Overly dry matrix due to excessive purge spin time	Check centrifuge settings
	Overly dry matrix due to excessive g-force	Check centrifuge settings
	Sample size ≤10 µl	Supplement with 10 µl deionized water
		Pipette 10 µl of deionized water onto top of matrix bed and spin for 2 min at 750 x g to increase recovery
High filtrate volume	Insufficient purge spin	Check centrifuge settings
	Insufficient g-force	Check centrifuge settings
	Sample size = 50 µl	Purge-spin for at least 5 min
Low signal intensity	Low DNA concentration due to poor cycle sequencing reaction	Check cycling parameters
	Overdrying of sample	Reduce drying time and temperature
	Insufficient resuspension	Increase resuspension time
High number of ambiguities	Low signal intensity	Refer to "low signal intensity" section
Dye terminator peaks (dye blobs)	Samples loaded along wall of well	Deposit sample directly onto center of resin bed
	Excessively wet resin bed	Spin for recommended time and at recommended g-force

Section 7. Storage Conditions

Store at 4°C. Do not freeze. Shelf life is 6 months at 4°C.

Section 8. Ordering Information

732-6500 SEQueaky Kleen H_2O™ Dye Terminator Removal Kit, 2 x 96 plates

732-6262 Quantum Prep™ Microplate Sealing Tape, 20 seals

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