

---

# SEQueaky Kleen H<sub>2</sub>O™ Dye Terminator Removal Kit

Instruction Manual

Catalog Number

732-6500

For technical service  
call your local Bio-Rad office or  
in the U.S. call 1-800-4BIORAD  
(1-800-424-6723)

On the Web at <http://www.discover.bio-rad.com>

**BIO-RAD**

# Table of Contents

<b>Section 1. Introduction .....</b>	<b>1</b>
<b>Section 2. Kit Components .....</b>	<b>1</b>
<b>Section 3. Instructions for Use .....</b>	<b>2</b>
<b>Section 4. Quick Guide .....</b>	<b>3</b>
<b>Section 5. Optimal Filtrate Range.....</b>	<b>4</b>
<b>Section 6. Troubleshooting Guide.....</b>	<b>5</b>
<b>Section 7. Storage Conditions .....</b>	<b>6</b>
<b>Section 8. Ordering Information .....</b>	<b>6</b>

# Section 1. Introduction

The SEQueaky Kleen H<sub>2</sub>O™ 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 Applied Biosystems

## Section 3. Instructions for Use

1. Remove sealing tape from the top and bottom of the SEQueaky Kleen H<sub>2</sub>O plate and cover with the lid of a collection plate.
2. Place the SEQueaky Kleen H<sub>2</sub>O 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.

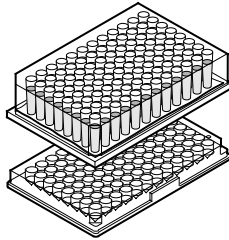
4. Remove the plate assembly from the centrifuge. Discard both the lid and collection plate. Transfer the SEQueaky Kleen H<sub>2</sub>O plate onto a clean collection plate.
5. Pipet the cycle sequencing reaction samples (≤50 µl) onto the center of the matrix bed in each well. Avoid penetration of the 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<sub>2</sub>O 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<sub>2</sub>O filter plate with lid on top of collection plate

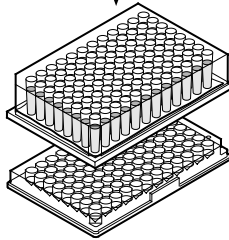


Place into tabletop centrifuge with microplate carrier rotor

Centrifuge 2 min at 750 x g to remove residual buffer



Change collection plate

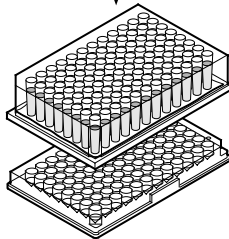


Load sequencing reactions (≤ 50 µl)

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.

<b>Sample volume</b>	<b>Expected filtrate volume</b>	<b>Comments</b>
10 $\mu$ l	12–15 $\mu$ l	Increase spin if filtrate is <10 $\mu$ l
20 $\mu$ l	18–22 $\mu$ l	Increase spin if filtrate is <15 $\mu$ l
50 $\mu$ l	30–35 $\mu$ l	Increase spin if filtrate is <25 $\mu$ l

## Section 6. Troubleshooting Guide

<b>Problem</b>	<b>Possible Cause</b>	<b>Possible Solutions</b>
<b>Low filtrate volume</b>	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 $\leq 10 \mu\text{l}$	Supplement with $10 \mu\text{l}$ deionized water  Pipette $10 \mu\text{l}$ of deionized water onto top of matrix bed and spin for 2 min at $750 \times g$ to increase recovery
<b>High filtrate volume</b>	Insufficient purge spin	Check centrifuge settings
	Insufficient g-force	Check centrifuge settings
	Sample size = $50 \mu\text{l}$	Purge-spin for at least 5 min
<b>Low signal intensity</b>	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
<b>High number of ambiguities</b>	Low signal intensity	Refer to “low signal intensity” section
<b>Dye terminator peaks (dye blobs)</b>	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<sub>2</sub>O™ Dye Terminator Removal Kit, 2 x 96 plates

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



