SEQueaky Kleen™ H_2O Adaptors

Instruction Manual

Catalog
732-6510

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

On the Web at discover.bio-rad.com

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Table of Contents

Section 1
Introduction

Designed specifically for use with the SEQueaky Kleen™ H_2O dye terminator removal kit, SEQueaky Kleen H_2O adaptors help streamline the dye terminator removal process. The adaptors align and hold the purification plates above 96-well sequencer injection plates, allowing the direct collection of purified sample into the injection plates. The injection plates can then be placed directly into various capillary fluorescent sequencers, where the labeled fragments are electrokinetically injected into the capillaries, electrophoretically separated, and analyzed. The adaptors are compatible with four common styles of injection plates (Table 1), each used in a specific multichannel capillary sequencer.

Table 1. Injection plates with compatible sequencers.

Injection Plate	Instrument
Half-skirt	ABI PRISM 3100 genetic analyzer ABI PRISM 3700 DNA analyzer ABI PRISM 3730(xl) DNA analyzer
Unskirted	MegaBACE 1000 DNA analysis system
Full-skirt	MegaBACE 500 DNA analysis system MegaBACE 1000 DNA analysis system BaseStation DNA fragment analyzer
Segmented	CEQ 2000XL DNA analysis system CEQ 8000 genetic analysis system

Section 2
Components

SEQueaky Kleen H_2O adaptors contain the following components:

SEQueaky Kleen H_2O adaptor base	2
SEQueaky Kleen H_2O adaptor bracket with removable handle	2
Instruction Manual	1
Protocol Overview	1

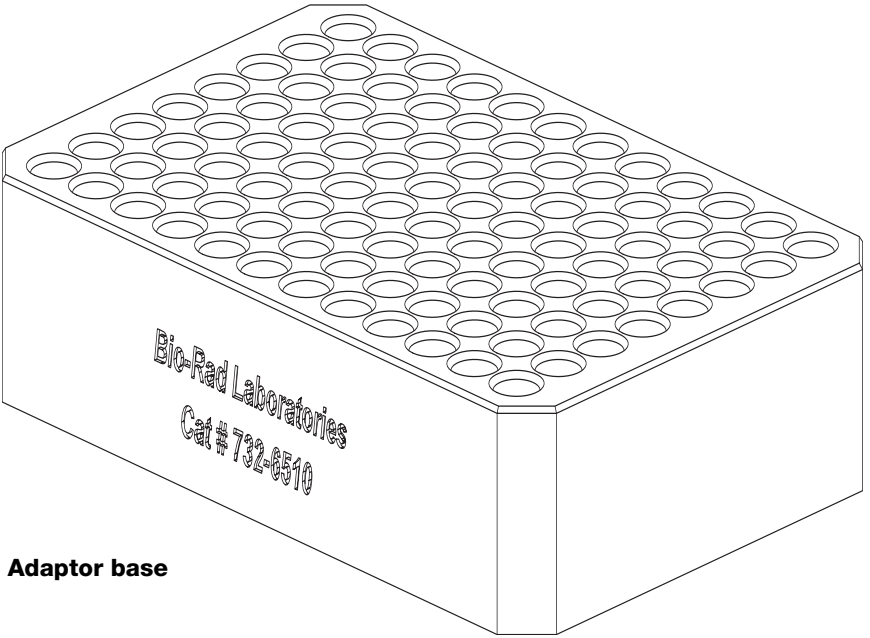
Section 3
Storage Conditions

Store at room temperature. The SEQueaky Kleen H_2O adaptors should be rinsed with deionized water and then dried with a paper towel periodically.

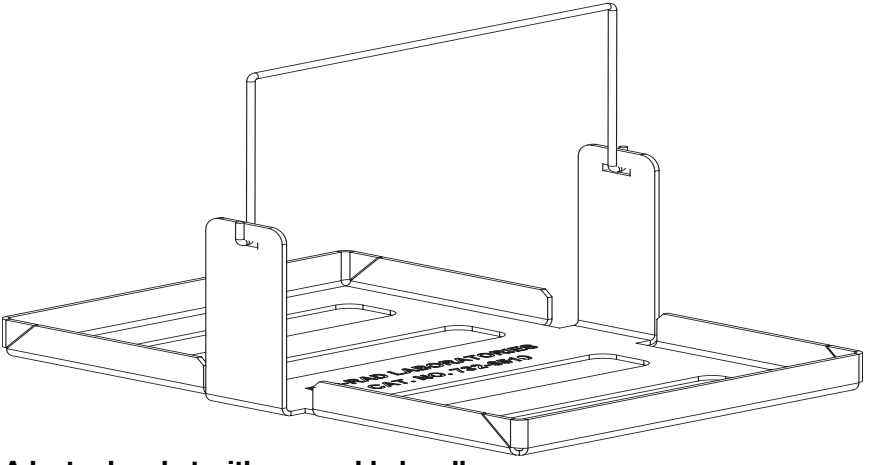
Section 4
Necessary Supplies

Equipment and supplies to be provided by the customer:

- SEQueaky Kleen H_2O dye terminator removal kit
- Sequencer injection plates
- Centrifuge with swinging bucket rotor and appropriate microplate carrier (see Section 5)



Adaptor base



Adaptor bracket with removable handle

Fig. 1 SEQueaky Kleen H_2O adaptor components

Section 5
Recommended Centrifuges and Rotors

For compatibility of other centrifuges, please contact Technical Support at 1-800-4BIORAD (outside the US, 510-741-1000).

Manufacturer	Centrifuge	Rotor
Beckman Coulter	Allegra 6	GH3.8 PTS-2000
	Allegra 25R	S-5700
	Avanti J-20 XP	JS-5.3
	Avanti J-25/30I	JS-5.9
	Allegra 21	S-2096
Brinkmann/Eppendorf	5804/5804R 5810/5810R	A-2-MTP A-2-DWP
Kendro/Sorvall	Legend T/RT	TTH-750
	Megafuge 1.0/1.0R	75002704

Section 6
Instructions for Use

1. Remove the sealing tapes from the top and bottom of the SEQueaky Kleen H_2O plate.
2. Place the SEQueaky Kleen H_2O plate on top of a collection plate. Transfer this assembly into the microplate carrier of a centrifuge. The brake should be set at maximum.

   Note: The SEQueaky Kleen H_2O adaptor is **NOT** used in this step.
3. Centrifuge for 2 min at 750 x g to purge residual water from the matrix beds. Begin timing as soon as centrifugation begins. Disregard the short time required for the rotor to stop rotating.
4. Remove the plate assembly from the centrifuge. Discard the collection plate.
5. Place an injection plate into the adaptor base and insert both into the adaptor bracket. Load a SEQueaky Kleen H_2O plate on top of the injection plate, aligning the orifices on the underside of the SEQueaky Kleen H_2O plate with the wells of the injection plate.
6. 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 a sample between the matrix bed and the wall of the well.

   Note: For 10 µl samples, we recommend adding 10 µl deinonized 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.
7. Transfer the entire assembly into a centrifuge carrier.
8. Centrifuge for 2 min at 750 x g again to recover the purified samples. Begin timing as soon as centrifugation begins.
9. After centrifugation, remove the assembly from the centrifuge carriers. Samples collected in the injection plate can be subjected directly to DNA sequence analysis by a multichannel capillary sequencer.

Section 7
Protocol Overview

SAMPLE PREPARATION

SEQueaky Kleen™ H_2O Adaptors

- Place SEQueaky Kleen H_2O plate on top of collection plate.
  Place into tabletop centrifuge with correct microplate carrier rotor.
- Centrifuge 2 min at 750 x g to remove excess water from matrix. Discard collection plate.
- Insert injection plate into adaptor base and place both into bracket. Place SEQueaky Kleen H_2O plate on top of injection plate.
  Load sequencing reactions (≤50 µl).
- Place assembly into tabletop centrifuge with correct microplate carrier rotor.
  Centrifuge 2 min at 750 x g. Dye terminators, low molecular weight species, and salts are retained in SEQueaky Kleen H_2O plate.
- The purified DNA is ready for direct placement into sequencer.

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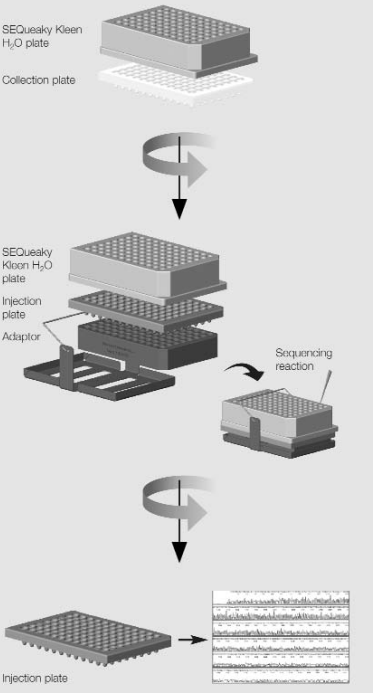

Section 8
Optimal Filtrate Volume Range

The following table indicates the range of optimal filtrate volumes recovered when purifying different samples.

Sample Volume	Expected Filtrate Volume
10 µl	12–15 µl
20 µl	15–22 µl
50 µl	30–35 µl

Section 9
Troubleshooting Guide

Problem	Possible Cause	Possible Solutions
Low filtrate volume	Overly dry matrix due to excessive purge-spin	Check centrifuge setting for spin time and/or g-force
	Sample size ≤10 µl	Supplement with 10 µl deionized water
		After initial purge-spin, pipet 10 µl of deionized water on top of matrix bed prior to sample loading
High filtrate volume	Insufficient purge-spin	Check centrifuge setting for spin time and/or g-force
	Excessive collection spin	Check centrifuge setting for spin time and/or g-force
	Adaptor used in the purge-spin	Use collection plate ONLY in purge-spin
	Sample size ≥ 50 µl	Purge-spin for at least 5 min

Problem	Possible Cause	Possible Solutions
Low signal intensity	Low DNA concentration due to poor cycle sequencing reaction	Check cycle sequencing parameters
	Low DNA concentration due to high filtrate volume	Refer to "High filtrate volume" section
	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	Purge-spin for recommended time and at recommended g-force
Irregular well-to-well sample volume and inconsistent sequencing data quality	Cross-contamination between injection plate wells	Make sure the SEQueaky Kleen H_2O plate is aligned and in contact with the injection plate

Section 10
Ordering Information

732-6500 SEQueaky Kleen H_2O Dye Terminator Removal Kit, 2 x 96-well dye terminator removal plates, 4 collection plates

732-6505 SEQueaky Kleen H_2O Bulk Kit, 50 x 96-well dye terminator removal plates, 100 collection plates

732-6510 SEQueaky Kleen H_2O Adaptors, set of 2 adaptor bases and brackets with handles

	Injection Plates			
	Half-skirt	**Unskirted**	**Full-skirt**	**Segmented**
Starter kit*	732-6530	732-6531	732-6532	732-6533
Refill kit**	732-6540	732-6541	732-6542	732-6543
50 pack***	732-6520	732-6521	732-6522	732-6523

*Starter kit includes set of 2 SEQueaky Kleen H_2O adaptors, 50 SEQueaky Kleen H_2O dye terminator removal plates, 50 injection plates, 50 collection plates.

**Refill kit includes 50 SEQueaky Kleen H_2O dye terminator removal plates, 50 injection plates, 50 collection plates.

***50 pack includes only injection plates.

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