

SAMPLE PREPARATION

SEQueaky Kleen™ H₂O Kit

Features of the SEQueaky Kleen H₂O Kit:

- Parallel processing of hundreds of sequencing reactions simultaneously
- 2-min spin separation, significantly faster than ethanol precipitation
- Elimination of “dye blobs”
- Effective removal of fluorescently labeled terminators for improved resolution and base calling
- Increased recovery of target DNA

The Water-Based High-Throughput Dye Terminator Removal System

Introduction

Over the last 5 years, automated DNA sequencing has evolved to meet the demand for dramatically increased throughput. To meet the needs of production laboratories, automated capillary fluorescent sequencing is now the standard for high-throughput platforms. With features including smaller starting sample volumes, electrokinetic sample uptake, and faster run times, sample purity criteria have become more stringent.

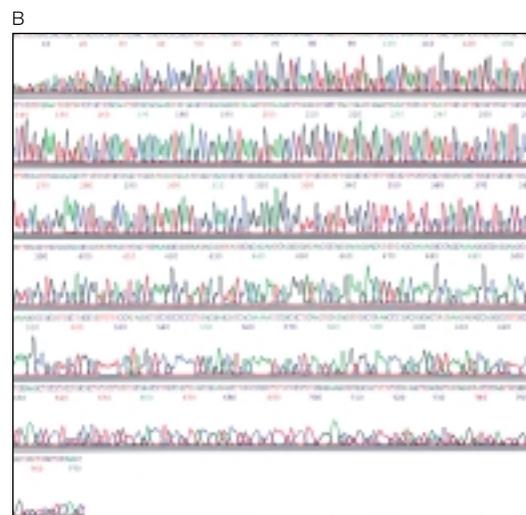
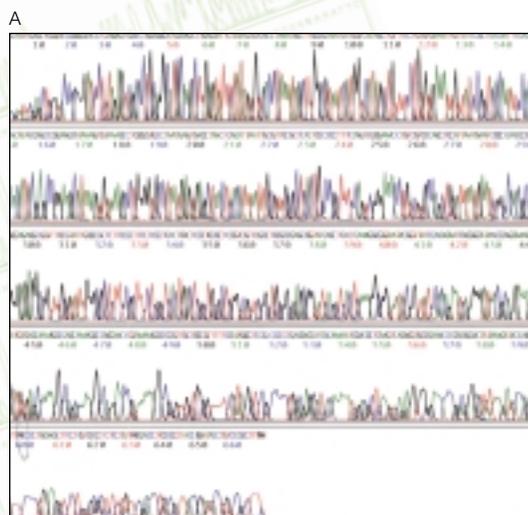
Automated capillary-based sequencing works by incorporating a capillary to hold the gel matrix, instead of a traditional slab-gel apparatus. Although this new technology offers high sensitivity along with higher throughput sample processing, it also requires a higher level of sample purity. Samples must be free of unincorporated dye terminators and other contaminants, including salts. Cleanup of cycle sequencing reactions must yield a highly purified product.



Dye terminator removal is one of the paramount cleanup objectives before sample addition onto either a slab-gel or capillary automated sequencer. The SEQueaky Kleen H₂O dye terminator removal kit allows up to 96 samples to be processed in parallel, and has been optimized for the removal of BigDye® terminators in pure water.

High-Throughput Processing

The SEQueaky Kleen H₂O kit has a new proprietary size exclusion matrix, fully hydrated and slurried in pure water and prepacked into a 96-well filter plate. Much easier, faster, and more reliable to use than traditional ethanol precipitation methods, SEQueaky Kleen H₂O minimizes handling and delivers purified samples ready to use into a 96-well microtiter plate.



Plasmid samples were purified with the SEQueaky Kleen H₂O dye terminator removal kit and sequenced on A, an ABI PRISM 377* DNA sequencer; B, an ABI PRISM 3700* DNA sequencer.

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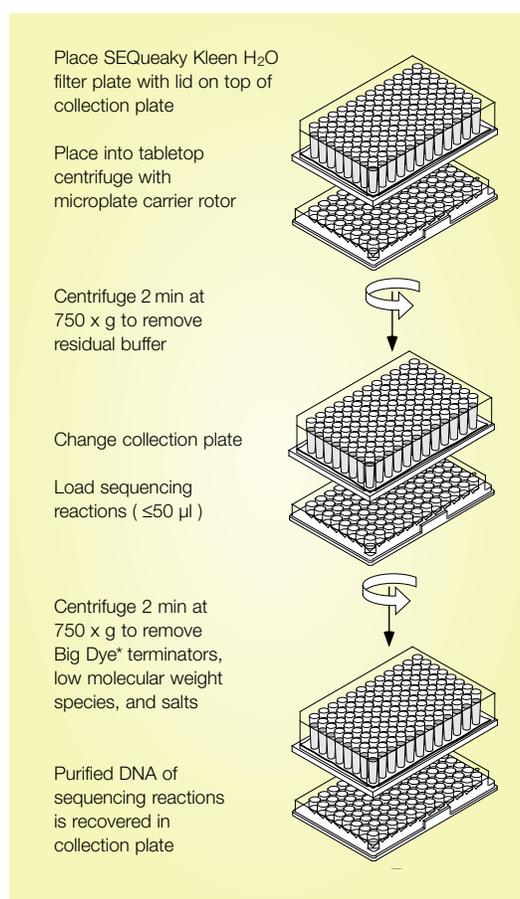
Using only a 2 min centrifugation, up to 96 different samples are simultaneously purified of any contaminating dye terminators and salts in water. Purified samples can be used directly in any downstream sequencing application and are ideally suited for capillary sequencing platforms.

Performance

The new proprietary gel matrix offers size exclusion chromatography in a fast and easy-to-use format. The pure water eluent allows the purified sample to be used in a capillary sequencing platform, without precipitations or other desalting steps.

Performance criteria of the SEQueaky Kleen H₂O kit include improved fragment recovery that provides higher signal intensities and longer sequence read lengths compared to traditional ethanol precipitation and other gel filtration products. Ready-to-use samples can be loaded on any commercially available DNA sequencer, including both slab gel and capillary electrophoresis platforms.

A cost-effective method for dye terminator removal, SEQueaky Kleen H₂O routinely and reliably produces the longest reads and fewest ambiguities in a high-throughput format using a variety of templates and primers.



Specifications

Purification method	Centrifuge-assisted size exclusion chromatography
Sample volume	10–50 µl (smaller sample volumes can also be accommodated; see manual for specific protocols)
Stability	6 months at 4°C; do not freeze
Recommended centrifuges	Beckman GH 3.8 horizontal rotor and MicroPlus [®] carrier (362394), Sorvall [®] H1000B swinging bucket rotor and a microplate carrier (11093), Eppendorf centrifuge 5804. For other centrifuges, please consult manufacturer for specific rotor
Compatibility	ABI [®] PRISM 3700 DNA analyzer sequencer ABI PRISM 3100 DNA analyzer sequencer ABI PRISM 377 DNA analyzer APB MegaBACE [®] 1000 DNA sequencing system Beckman CEQ [®] 2000XL DNA analysis system

Ordering Information

Catalog #	Description
732-6500	SEQueaky Kleen H ₂ O Kit, includes 2 dye terminator removal plates, 4 collection plates, quick guide and protocol manual.

High-throughput quantities and bulk pricing are available upon request.

* ABI PRISM and BigDye are trademarks of Applied Biosystems. MegaBACE is a trademark of Amersham Pharmacia Biotech. MicroPlus is a trademark of Beckman Coulter, Inc. Sorvall is a trademark of E. I. du Pont de Nemours and Company.

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