SEQUENCING PRODUCT PURIFICATION MAGNETIC BEADS

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-007
Product Name	Sequencing Product Purification Magnetic Beads
Category	Sanger Cleanup Reagent
Pack Size	10,000T/tube
Regulatory Status	For Research Use Only (RUO)
OEM Reference	Contact techsupport@enzovera.com
Version	1.0
Issue Date	2026-05-07

2. INTENDED USE

This product is intended for the purification of Sanger cycle sequencing extension products prior to capillary electrophoresis analysis. The silica-hydroxyl magnetic beads selectively bind DNA while removing unincorporated fluorescent dye terminators, salts, enzymes, and short primer fragments that interfere with sequencing read quality. The purified DNA is suitable for direct injection on capillary electrophoresis instruments including the ABI 3730xl, 3500, and compatible sequencing platforms. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Sequencing Purification Magnetic Beads	60 mL	15-25°C
85% Ethanol Wash Solution	100 mL	15-25°C
Resuspension Buffer (0.1 mM EDTA)	50 mL	15-25°C
96-well Magnetic Separation Plate	2 plates	15-25°C
Product Insert	1 booklet	Room temperature

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Magnetic separation stand or plate compatible with sample vessel format
- Freshly prepared 70% or 80% ethanol for washing steps
- Nuclease-free water or TE buffer for elution
- Pipettes and aerosol-resistant pipette tips (10 µL, 20 µL, 200 µL, 1000 µL)
- PCR plates, tubes, or strip tubes compatible with magnetic separation
- Vortex mixer or plate shaker for bead resuspension
- Centrifuge for brief spin-down of samples (optional but recommended)
- Thermal cycler or heat block for Sanger sequencing reaction setup

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	Brown magnetic bead suspension
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

SEQUENCING PRODUCT PURIFICATION PROTOCOL

Silica-Hydroxyl Magnetic Beads for Sanger Cycle Sequencing Cleanup

PRODUCT OVERVIEW

Enzovera Sequencing Product Purification Magnetic Beads are designed for high-throughput removal of unincorporated fluorescent dye terminators, salts, dNTPs, and short oligonucleotide fragments from Sanger cycle sequencing extension products prior to capillary electrophoresis. The silica-hydroxyl surface chemistry selectively binds sequencing DNA fragments while leaving free dye terminators and small molecules in solution for efficient removal. This protocol achieves greater than or equal to 95% dye terminator removal efficiency and provides clean sequence reads with minimal dye blob artifacts.

APPLICATIONS

- Cleanup of BigDye Terminator v1.1 and v3.1 cycle sequencing reactions

- Purification of dGTP BigDye Terminator sequencing products

- Removal of unincorporated fluorescent ddNTPs from extension reactions

- Preparation of sequencing DNA for ABI 3730xl, 3500, and SeqStudio capillary electrophoresis systems

- High-throughput 96-well and 384-well plate processing

- Automated liquid handling workstation protocols

MATERIALS REQUIRED

Provided:

- Sequencing Product Purification Magnetic Beads (stored at 2-8°C)

Required but not provided:

- 85% (v/v) ethanol, freshly prepared (200 proof absolute ethanol diluted with nuclease-free water)

- Hi-Di Formamide (Applied Biosystems or equivalent deionized formamide)

- Magnetic separation device (96-well plate magnet, tube magnet, or automated platform)

- Nuclease-free 1.5 mL microcentrifuge tubes or 96-well PCR plates

- Pipettes and aerosol-barrier pipette tips (10 µL, 20 µL, 200 µL, 1000 µL)

- Vortex mixer

- Microcentrifuge (optional, for tube format)

- Thermal cycler or heat block capable of 95°C (optional, for denaturation)

IMPORTANT NOTES BEFORE STARTING

- Allow magnetic beads to equilibrate to room temperature (18-25°C) for at least 30 minutes before use

- Vortex bead suspension vigorously for 30 seconds immediately before each use to ensure complete resuspension

- Prepare fresh 85% ethanol daily for optimal performance

- Do not allow beads to dry completely during washing steps as this reduces DNA recovery

- Use the same pipette tip throughout magnetic bead manipulation steps to minimize DNA loss

- For automated platforms, optimize bead drying time based on ambient humidity conditions

PROTOCOL FOR 96-WELL PLATE FORMAT (RECOMMENDED)

1. Transfer 10 µL of completed cycle sequencing reaction to each well of a 96-well PCR plate. If reaction volume is less than 10 µL, add nuclease-free water to bring total volume to 10 µL.

2. Vortex the bottle of Sequencing Product Purification Magnetic Beads for 30 seconds to ensure homogeneous suspension. Beads settle rapidly during storage.

3. Add 10 µL of resuspended magnetic beads to each sequencing reaction well. The bead-to-sample ratio is 1:1 (v/v) for optimal purification.

4. Pipette mix by aspirating and dispensing 15 µL at least 10 times, or seal plate and vortex at medium speed for 10 seconds. Ensure complete mixing of beads and sequencing reaction.

5. Incubate the plate at room temperature for 5 minutes to allow DNA binding to the silica-hydroxyl bead surface. During this time, unincorporated dye terminators remain in solution.

6. Place the 96-well plate on a magnetic separation device. Allow beads to collect at the magnet side for 2 minutes until the supernatant appears clear and colorless.

7. While plate is on the magnet, carefully remove and discard the supernatant containing unincorporated dyes and salts using a multichannel pipette. Aspirate from the side opposite the bead pellet. Do not disturb the beads.

8. Keep the plate on the magnetic separation device. Add 30 µL of freshly prepared 85% ethanol to each well without disturbing the bead pellet.

9. Incubate on magnet for 30 seconds. The ethanol wash removes residual salts and dye terminators while DNA remains bound to beads.

10. Carefully remove and discard the ethanol wash without disturbing the beads. Aspirate from the side opposite the magnetic pellet.

11. Repeat the ethanol wash (steps 8-10) for a second wash. Two washes are critical for achieving greater than 95% dye removal efficiency.

12. After removing the second ethanol wash, briefly pulse centrifuge the plate at 200 x g for 5 seconds to collect residual eth

8. EXPECTED RESULTS

When used according to protocol, these magnetic beads will remove ≥95% of unincorporated dye terminators from Sanger sequencing extension products while retaining full-length sequences, resulting in clean electropherograms with minimal dye blob artifacts at the injection origin and improved basecalling accuracy beyond 800 bp read length. Purified samples analyzed on capillary electrophoresis systems such

as the ABI 3730xl should exhibit signal-to-noise ratios comparable to CleanSEQ performance, with sharp peak resolution and minimal background fluorescence across all four dye channels. Recovery of extension products ≥100 bp should exceed 85%, enabling reliable sequence data from both standard and difficult templates.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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