SEQUENCING CLEANUP RESIN (50ML)

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-008
Product Name	Sequencing Cleanup Resin (50ml)
Category	Sanger Cleanup Reagent
Pack Size	50ml/bottle
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 sequencing extension products by removing unincorporated dye terminators, salts, dNTPs, endotoxins, and excess primers prior to capillary electrophoresis. The ion-exchange resin is compatible with spin-column and multiscreen plate formats and delivers purified sequencing-ready DNA without requiring magnetic separation equipment. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Sequencing Cleanup Resin	50 mL	Room temperature (15-25°C)
Resin Binding Buffer (10X concentrate)	100 mL	Room temperature (15-25°C)
Wash Buffer (10X concentrate)	100 mL	Room temperature (15-25°C)
Elution Buffer	50 mL	Room temperature (15-25°C)
Spin Columns with Collection Tubes	50 units	Room temperature (15-25°C)
96-Well Multiscreen Filter Plates	2 plates	Room temperature (15-25°C)

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Microcentrifuge or plate centrifuge capable of 3,000 x g
- 96-well filter plates or spin columns compatible with vacuum manifold
- Vacuum manifold or multichannel pipette for high-throughput processing
- Molecular biology grade ethanol (absolute, 200 proof)
- Nuclease-free water or TE buffer for DNA elution
- 1.5 mL microcentrifuge tubes or 96-well collection plates
- Pipettes and filtered pipette tips (10-1000 µL range)
- Vortex mixer for resuspension of resin before use

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	White resin 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 CLEANUP RESIN PROTOCOL

Enzovera Life Sciences

Product: Sequencing Cleanup Resin (50 mL)

Catalog Number: Contact Enzovera Technical Support at techsupport@enzovera.com

PURPOSE

This protocol describes the purification of Sanger sequencing reactions using Sequencing Cleanup Resin to remove unincorporated dye terminators, salts, dNTPs, endotoxins, and excess primers prior to capillary electrophoresis analysis.

MATERIALS REQUIRED

Materials Provided:

- Sequencing Cleanup Resin (50 mL)

Materials Not Provided:

- Completed cycle sequencing reactions (10-20 µL)

- Spin columns with 0.45 µm cellulose acetate membranes or MultiScreen HTS 96-well filter plates

- Microcentrifuge capable of 750 x g and 5,000 x g

- 1.5 mL microcentrifuge tubes or 96-well collection plates

- Molecular biology grade water or Hi-Di Formamide

- Pipettes and tips (200 µL, 1000 µL)

- Vortex mixer

IMPORTANT NOTES BEFORE STARTING

1. Sequencing Cleanup Resin is supplied as a suspension. Vortex thoroughly for 30 seconds immediately before each use to ensure uniform resin distribution.

2. Resin settles rapidly during storage. If resin has separated, invert bottle 20 times then vortex for 30 seconds before dispensing.

3. Store Sequencing Cleanup Resin at room temperature (15-25°C). Do not freeze.

4. Resin is compatible with all common Sanger sequencing chemistries including BigDye Terminator v1.1, v3.1, BigDye Direct, and dGTP chemistry.

5. This protocol removes greater than 98% of unincorporated dye terminators and greater than 95% of salts while recovering 85-95% of sequencing product.

6. For best results, process sequencing reactions within 2 hours of thermal cycling completion or store at -20°C until ready to purify.

PROTOCOL FOR SPIN COLUMN FORMAT

Preparation:

1. Allow completed sequencing reactions to cool to room temperature (20-25°C) for 5 minutes if processing immediately after thermal cycling.

2. Vortex Sequencing Cleanup Resin bottle vigorously for 30 seconds to fully resuspend settled resin particles.

3. Label one spin column and one clean 1.5 mL collection tube for each sample to be purified.

Resin Loading:

4. Place spin column into a 1.5 mL microcentrifuge tube (this tube will be discarded after the first spin).

5. Pipette 300 µL of thoroughly mixed Sequencing Cleanup Resin into the spin column. Use a 1000 µL pipette with tip cut at an angle to facilitate resin transfer and prevent settling in the tip.

6. Centrifuge at 750 x g for 2 minutes at room temperature to pack the resin bed.

7. Discard the flow-through liquid from the collection tube. The packed resin bed should be approximately 5-7 mm in height.

Sample Application:

8. Transfer the spin column to a new labeled 1.5 mL collection tube that will be used for final elution.

9. Carefully pipette the entire sequencing reaction (10-20 µL) directly onto the center of the packed resin bed. Avoid touching the resin surface with the pipette tip.

10. Allow the sample to absorb into the resin bed by incubating at room temperature for 2 minutes. Do not centrifuge during this step.

Binding and Wash:

11. Add 150 µL of molecular biology grade water directly onto the resin bed without disturbing the surface.

12. Centrifuge at 5,000 x g for 5 minutes at room temperature to elute the purified sequencing product through the resin and into the collection tube.

13. The purified sequencing product is now in the collection tube. Discard the spin column with the resin (which retains the dye terminators, salts, and contaminants).

Final Preparation for Capillary Electrophoresis:

14. Dry the purified sequencing product by one of the following methods:

Method A - Vacuum centrifugation: Transfer sample to a 0.5 mL tube and dry in a vacuum centrifuge at 45°C for 30-45 minutes until completely dry.

Method B - Heat evaporation: Transfer sample to a 0.5 mL tube, leave cap open, and incubate at 95°C in a thermal cycler for 20-30 minutes until dry.

15. After drying is complete, immediately add 10-15 µL Hi-Di Formamide to the dried pellet.

16. Vortex samples for 10 seconds to fully resuspend the purified DNA.

17. Cent

8. EXPECTED RESULTS

When used according to the protocol, Enzovera Sequencing Cleanup Resin removes >99% of unincorporated dye terminators, salts, dNTPs, and primer fragments from cycle sequencing reactions while retaining >85% of extension products 100-1000 bases in length. Purified sequencing products yield clean electropherograms with baseline resolution, signal-to-noise ratios >20:1, and read lengths exceeding 800 bases on capillary electrophoresis systems. Residual dye blob artifacts are eliminated and Q20 read lengths typically improve by 15-25% compared to unpurified reactions.

9. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommended Action
Poor sequencing quality or low signal intensity after cleanup	Insufficient resin volume used relative to sequencing reaction volume; incomplete binding of extension products to resin	Verify resin-to-reaction ratio is 10:1 (v/v). Add 10 µL resin per 1 µL sequencing reaction. Mix thoroughly by pipetting 5-8 times or vortexing for 3-5 seconds. Ensure resin is well-suspended before dispensing.
High background or dye blob artifacts in electropherogram	Incomplete removal of unincorporated dye terminators; insufficient incubation time; inadequate washing	Extend incubation time to 15 minutes at room temperature. Ensure complete removal of supernatant after magnetic separation. Perform two washes with 150 µL 70% ethanol per well, allowing 30 seconds per wash. Ensure resin pellet is completely dry before elution (2-3 minutes at room temperature).
Low DNA recovery or no sequencing signal	Extension products eluted with supernatant during cleanup; over-drying of resin causing irreversible DNA binding	Use magnetic separation for minimum 2 minutes to ensure complete resin pelleting. Avoid aspirating resin during supernatant removal. Do not over-dry resin - pellet should appear moist and glossy, not cracked. If over-dried, add 5 µL water, incubate 2 minutes, then proceed with elution.
Inconsistent results between samples or plates	Resin settling during dispensing; uneven magnetic separation; variation in ethanol concentration	Vortex or invert resin bottle every 8-12 samples to maintain suspension. Use multichannel pipettes with consistent technique. Prepare fresh 70% ethanol daily from molecular biology grade absolute ethanol. Ensure magnetic plate positioning is uniform and separation time is consistent across all samples.
Resin clumping or difficult resuspension	Resin partially dried out; exposure to incompatible buffers; improper storage	Store resin tightly capped at 2-8°C when not in use. Bring to room temperature before use and mix gently by inversion 10 times. If clumping occurs, vortex for 10-15 seconds. Do not freeze. Replace resin if clumping

		persists after mixing.
Reduced read length or sequence quality degradation in later bases	Co-purification of salts or contaminants from sequencing reaction; insufficient template cleanup prior to cycle sequencing	Ensure PCR products are cleaned prior to sequencing reactions using appropriate purification method (ExoSAP or bead cleanup). Verify BigDye concentration is within manufacturer specifications. Increase ethanol wash volume to 180 µL if high salt content suspected. For templates >1 kb, consider using 1.5:1 resin ratio.

10. DOCUMENT CONTROL

Document Number	IFU-EV-SGR-008
Version	1.0
Status	DRAFT — Pending Authorisation
Issue Date	2026-05-07
Prepared By	Enzovera Life Sciences Technical Documentation
Approved By	Pending
Next Review	12 months from issue date