

INSTRUCTIONS FOR USE

Sanger Sequencing Reagents

Sequencing Cleanup Resin

Post-Reaction Cleanup Reagent

Catalog No.: EV-SGR-008

Version: 1.1 | For Research Use Only

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1. Product Information

Product name	Sequencing Cleanup Resin
Catalog number	EV-SGR-008
Intended use	Ion-exchange resin for removal of dye terminators, salts, dNTPs, endotoxins, and primer fragments from Sanger sequencing reactions prior to capillary electrophoresis, compatible with spin-column and 96-well multiscreeen plate formats without requiring magnetic separation equipment.
Storage conditions	Store at 2–8°C. Resin settles during storage — invert gently to resuspend before use. Do not freeze. Shelf life: 12 months from date of manufacture.
Regulatory status	For Research Use Only (RUO). Not for diagnostic or therapeutic use.

2. Product Description

Enzovera Sequencing Cleanup Resin (EV-SGR-008) is an ion-exchange resin for removal of dye terminators, salts, dNTPs, endotoxins, and primer fragments from Sanger cycle sequencing reactions prior to capillary electrophoresis injection. The resin eliminates the need for magnetic separation equipment, making it the cleanup method of choice for laboratories without dedicated magnetic plate stations or for protocols where spin-column or 96-well multiscreeen plate workflows are preferred. It is supplied as a 50 mL ready-to-use suspension and is compatible with both individual spin-column and high-throughput 96-well multiscreeen plate formats, delivering CE-ready sequencing product in a single-step, single-tube cleanup that requires approximately 10–15 minutes from reaction completion to injection-ready sample.

The resin operates on an ion-exchange principle: under binding conditions, negatively charged contaminants including free dye terminators, dNTPs, salts, and short primer fragments are retained on the positively charged resin matrix, while the longer extension products pass through in the flow-through fraction. This single-pass mechanism simultaneously removes all major classes of CE contaminants without requiring separate desalting or size-exclusion steps. Dye terminator removal exceeds 95% under standard conditions, and sequencing extension products are recovered with high yield, ready for direct resuspension in Hi-Di Formamide (EV-SGR-011) for CE injection.

EV-SGR-008 is compatible with all standard BigDye-format cycle sequencing chemistries, including VeraDye™ Terminator v3.1 Cycle Sequencing Kit (EV-SGR-002) and third-party BigDye-format reagents. It is suitable as a backup or alternative cleanup workflow alongside magnetic bead methods (EV-SGR-007) for laboratories running both formats, and for cleanup of sequencing reactions with elevated salt or endotoxin content from upstream template preparation steps.

3. General Usage Guidelines

- Store at 2–8°C with the cap tightly closed. Do not freeze — freezing causes irreversible changes to the resin matrix. Resin settles during storage; invert the bottle gently 20 times, then vortex for 30 seconds to fully resuspend before dispensing.
- Resuspend the resin immediately before each use — do not let it settle in the pipette tip between samples. For 96-well plate workflows, re-invert or re-vortex the stock bottle every 8–12 samples to maintain a uniform suspension throughout the dispensing process.

- Use a wide-bore or angle-cut pipette tip when dispensing resin to prevent clogging. Return the stock bottle to 2–8°C storage promptly after use. Discard any resin that shows persistent clumping, cloudiness, or discoloration that does not resolve on mixing.

4. Safety Precautions

Read the Safety Data Sheet (SDS) and follow all handling instructions before use. Wear appropriate personal protective equipment including laboratory coat, gloves, and protective eyewear when handling this product. If contact with skin or eyes occurs, wash immediately with large amounts of water and seek medical attention if irritation persists. Dispose of the product in accordance with applicable local, state, and federal regulations.

5. Usage Instructions

The following protocol describes cleanup of Sanger sequencing reactions using the spin-column format (individual tube processing). For 96-well multiscreen plate workflows, dispense resin into the filter plate wells and apply vacuum or centrifugation to collect cleaned products into a collection plate below. In all formats, ensure the resin is thoroughly resuspended immediately before dispensing.

Resin loading: Place a spin column into a 1.5 mL collection tube. Vortex the resin bottle for 30 seconds, then pipette 300 µL of well-mixed resin into the spin column using a wide-bore or angle-cut tip.

Centrifuge at $750 \times g$ for 2 minutes at room temperature to pack the resin bed; discard the flow-through. The packed bed height should be approximately 5–7 mm. Transfer the spin column to a new, labeled 1.5 mL collection tube. **Sample application:** Pipette the completed sequencing reaction (10–20 µL) directly onto the center of the packed resin bed. Allow the sample to absorb into the resin for 2 minutes at room temperature — do not centrifuge during this step. Add 150 µL of nuclease-free water to the resin bed without disturbing the surface.

Elution and CE preparation: Centrifuge at $5,000 \times g$ for 5 minutes at room temperature to elute the purified sequencing product through the resin into the collection tube. Discard the spin column — the resin retains the dye terminators, salts, and other contaminants. Dry the eluted product by vacuum centrifugation (45°C, 30–45 minutes) or heat evaporation (95°C, 20–30 minutes, cap open) until completely dry. Immediately resuspend the dried pellet in 10–15 µL of Hi-Di Formamide (EV-SGR-011). Vortex briefly, pulse-centrifuge, heat-denature at 95°C for 3 minutes, snap-chill on ice, and load onto the CE instrument.

6. Storage and Stability

Store at 2–8°C with the cap tightly closed. Do not freeze — freezing causes irreversible changes to the resin matrix and will render the bottle unusable. Shelf life is 12 months from the date of manufacture when stored at 2–8°C. Resin settles rapidly during storage; invert the bottle gently and vortex before each use. Opened bottles may be stored at 2–8°C until the expiration date provided the cap is kept closed between uses and the resin is not contaminated. Discard any bottle showing persistent clumping or discoloration that does not resolve on mixing.

7. Troubleshooting

Observation	Possible Cause	Corrective Action
Poor sequencing quality or weak signal after cleanup	Insufficient resin volume relative to sequencing reaction volume; resin not fully resuspended before dispensing	Verify 300 μ L of well-mixed resin is loaded per spin column. Vortex the resin bottle for 30 seconds immediately before dispensing. Pack the resin bed by centrifugation at 750 $\times$ g for 2 minutes before applying the sample.
Dye blob artifacts or elevated early-read background persist after cleanup	Incomplete dye terminator retention by the resin; insufficient sample absorption time before elution centrifugation	Allow the sequencing reaction to absorb into the packed resin bed for a full 2 minutes before adding water. Ensure the resin bed was properly packed (750 $\times$ g, 2 min) and is 5–7 mm in height before sample application.
Low DNA recovery or no sequencing signal after elution	Centrifuge speed too high during packing, pulling extension products through prematurely; product lost during drying; resin bed over-dried	Confirm packing centrifugation is at exactly 750 $\times$ g for 2 minutes. Monitor drying step closely — stop as soon as the pellet is fully dry. If over-dried, rehydrate with 5 μ L nuclease-free water, incubate 2 minutes, then resuspend in formamide.
Inconsistent cleanup results between samples in the same batch	Resin settling between samples during dispensing, causing variable bed volumes; uneven centrifuge rotor loading	Re-invert and vortex the resin bottle every 8–12 samples during dispensing. Use a balanced rotor. For 96-well plate format, use a plate centrifuge to ensure uniform spin. Confirm all columns are filled to the same resin volume before the packing spin.
Resin clumping or difficult resuspension from the bottle	Resin partially dried from repeated opening; bottle stored with cap loose; frozen and thawed	Store at 2–8°C with cap tightly closed. Invert 20 times then vortex for 10–15 seconds to break up clumps. Discard any bottle where clumping persists after vigorous mixing, or contact techsupport@enzovera.com.

8. Technical Support

For technical assistance with Sanger Sequencing Reagents products, please contact Enzoverta Life Sciences:

techsupport@enzovera.com

9. Legal Notice

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