INSTRUCTIONS FOR USE

Sanger Sequencing Reagents

Template Preparation Magnetic Beads v3 (Silica-Hydroxyl)

Template Preparation Reagent

Catalog No.: EV-SGR-009
Version: 1.1 | For Research Use Only
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1. Product Information

Product name	Template Preparation Magnetic Beads v3 (Silica-Hydroxyl)
Catalog number	EV-SGR-009
Intended use	Silica-hydroxyl magnetic beads for rapid purification of plasmid DNA and PCR products to generate sequencing-ready Sanger templates, removing primers, salts, enzymes, and nucleotides that interfere with cycle sequencing reactions and capillary electrophoresis.
Storage conditions	Store at 2–8°C. Do not freeze. Vortex briefly to resuspend before use. 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 Template Preparation Magnetic Beads v3 (EV-SGR-009) are silica-hydroxyl–surface magnetic beads developed for rapid purification of plasmid DNA and PCR products prior to Sanger sequencing. The beads bind double-stranded DNA under chaotropic binding conditions and release purified template in low-salt elution buffer, providing a high-throughput alternative to column-based miniprep and spin-column PCR cleanup workflows. Supplied at 10,000 reactions per tube, the product is sized for core facility and CRO sequencing operations running 96- and 384-well plate template preparation workflows. A typical 96-well template prep cycle completes in 30–45 minutes from cell lysate or PCR product to eluted template, replacing the 2–3 hour timeline of column-based miniprep workflows.

The v3 silica-hydroxyl formulation has been optimized for the dsDNA size range relevant to Sanger sequencing template preparation: plasmids up to approximately 20 kb (including standard cloning vectors, expression constructs, and BAC subclones), PCR products from 100 bp to 10 kb, and restriction fragments. The beads bind dsDNA in the presence of chaotropic salts, which drive DNA adsorption onto the silica-hydroxyl surface. Contaminating proteins, RNA, salts, dNTPs, and primers are removed in subsequent wash steps. Elution in low-salt buffer (10 mM Tris-HCl pH 8.0 or nuclease-free water) releases purified template ready for direct use in cycle sequencing reactions. Typical yields are 5–15 µg per mL of overnight bacterial culture for high-copy plasmids, with DNA recovery of 85–95% from PCR reactions. The bead format is fully compatible with all major automated liquid handling platforms, including Beckman Biomek, Hamilton STAR, Tecan Freedom EVO, and Eppendorf epMotion systems.

The beads are not optimized for genomic DNA preparation, where larger-fragment-binding chemistries are preferred, or for RNA preparation. The amplification product from the Phi29 Rolling Circle Amplification Kit (EV-SGR-012) is also compatible with elution buffer from these beads; however, RCA products do not require a template prep bead cleanup step and can proceed directly to cycle sequencing after dilution.

3. General Usage Guidelines

- Store at 2–8°C. Do not freeze — freezing causes irreversible bead aggregation that cannot be recovered by vortexing. Vortex vigorously for 30 seconds immediately before pipetting; beads settle rapidly and an incompletely resuspended stock will deliver inconsistent bead volumes across wells.

- Prepare fresh 80% ethanol wash solution on the day of use by diluting 200-proof absolute ethanol with nuclease-free water. Do not allow beads to dry completely during wash steps — over-drying reduces DNA recovery. Limit air-drying to 3–5 minutes; beads should appear matte but not cracked.
- The bead-to-sample volumetric ratio determines the size range of fragments captured. Use higher ratios (1.8×) for short fragments (100–300 bp) and lower ratios (1.0×) for large fragments (above 1 kb). Return the stock tube to 2–8°C refrigerated storage promptly after use.

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 PCR product cleanup in 96-well plate format (recommended for high-throughput use). For individual tube processing, scale volumes proportionally. For plasmid template preparation from bacterial lysate, follow the same binding-wash-elution workflow using the binding buffer supplied with the kit. Allow the bead stock to reach room temperature and vortex for 30 seconds before pipetting. Prepare fresh 80% ethanol on the day of use.

Binding: Transfer 10–50 µL of completed PCR reaction or plasmid lysate to each well. Add beads at the appropriate volumetric ratio for the target fragment size: 1.8× for 100–300 bp; 1.5× for 300–500 bp; 1.2× for 500–1,000 bp; 1.0× for fragments above 1 kb. Mix thoroughly by pipetting up and down 10 times or by vortexing at medium speed for 5 seconds. Incubate at room temperature for 5 minutes to allow DNA to bind to the silica-hydroxyl bead surface. Washing: Place the plate on a magnetic separation rack and allow beads to collect for 2–3 minutes until the supernatant is clear. Remove and discard the supernatant. Add 200 µL of fresh 80% ethanol without disturbing the bead pellet; incubate on the magnet for 30 seconds; remove and discard the ethanol. Repeat the ethanol wash once for a total of two wash cycles. Use a fine-tip pipette to remove any residual ethanol droplets after the second wash.

Elution: Allow the bead pellet to air-dry on the magnet with lids open for 3–5 minutes until residual ethanol has evaporated — do not over-dry. Remove the plate from the magnet and add 30–50 µL of nuclease-free water or 10 mM Tris-HCl pH 8.0 per well. Pipette mix to resuspend the beads. Incubate at room temperature for 2 minutes. Return the plate to the magnet for 2 minutes to re-pellet beads. Transfer the clear supernatant containing the purified template to a fresh plate or tubes for use in cycle sequencing reactions. Purified templates are directly compatible with VeraDye™ Terminator v3.1 Cycle Sequencing Kit (EV-SGR-002) and BigDye-format cycle sequencing protocols without further adjustment.

6. Storage and Stability

Store at 2–8°C. Do not freeze — freezing causes irreversible aggregation of the bead suspension and the tube must be discarded if frozen. Shelf life is 12 months from the date of manufacture when stored continuously at 2–8°C. Vortex briefly to resuspend settled beads before each use. Between uses,

return the stock tube to 2–8°C refrigerated storage promptly. The bead suspension is stable in the tube for the duration of the printed shelf life under recommended storage conditions.

7. Troubleshooting

Observation	Possible Cause	Corrective Action
Low DNA yield or weak sequencing signal from purified template	Beads over-dried during wash step, causing irreversible DNA binding; bead-to-sample ratio too high for the target fragment size; beads not fully resuspended during elution	Limit air-drying to 3–5 minutes (beads matte, not cracked). Verify the correct bead-to-sample ratio for the fragment size range. During elution, pipette mix thoroughly to resuspend beads fully and maximize release of bound DNA.
Residual primer or salt contamination in eluted template; poor sequencing read quality despite adequate template concentration	Ethanol wash concentration below 80%; ethanol wash not freshly prepared; residual ethanol from incomplete drying inhibiting downstream sequencing reaction	Prepare fresh 80% ethanol daily from absolute ethanol. Perform two complete wash cycles. Use a fine-tip pipette to remove residual ethanol droplets after the second wash. Ensure the bead pellet is fully dry (3–5 minutes open on magnet) before elution.
Inconsistent DNA recovery across wells in the same plate	Bead stock not fully resuspended before pipetting, resulting in bead-volume variation across the plate; uneven evaporation during drying step	Vortex the stock tube vigorously for 30 seconds immediately before pipetting. Use a multichannel pipette and pipette-mix all wells simultaneously. Minimize variation in drying time by keeping plate covered when not actively pipetting.
Beads do not pellet or pellet slowly on the magnetic rack; bead aggregation visible	Beads frozen and irreversibly aggregated; plate not seated flat on the magnet; incompatible plate geometry with the magnetic rack	Discard any bead stock that has been frozen — freeze-aggregated beads cannot be recovered. Ensure the plate is seated flat and in direct contact with the magnet surface. Allow up to 3 minutes for pelleting. If unresolved, contact techsupport@enzovera.com.

8. Technical Support

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

techsupport@enzovera.com

9. Legal Notice

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