5× SEQUENCING BUFFER

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-003
Product Name	5× Sequencing Buffer
Category	Sanger Sequencing Reagent
Pack Size	5ml, 30ml
Regulatory Status	For Research Use Only (RUO)
OEM Reference	Contact techsupport@enzovera.com
Version	1.0
Issue Date	2026-05-07

2. INTENDED USE

Enzovera 5× Sequencing Buffer is a highly optimized reaction buffer designed for use with dye terminator-based Sanger sequencing reactions on capillary electrophoresis systems. This buffer is formulated to stabilize sequencing reactions, enhance peak height uniformity across all four bases, and minimize background fluorescence for improved base-calling accuracy and extended read lengths. When diluted to 1× final concentration, it is fully compatible with BigDye chemistry and standard cycle sequencing protocols on ABI 3730xl, 3500, and other capillary sequencers. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
5× Sequencing Buffer	5 mL or 30 mL (as ordered)	-20°C
TRIS-HCl (pH 9.0)	400 mM (in 1× solution)	Component of buffer
Magnesium chloride ($MgCl_2$)	10 mM (in 1× solution)	Component of buffer
Detergent stabilizer	Proprietary concentration	Component of buffer
Product Insert	1 copy	Room temperature

4. MATERIALS REQUIRED BUT NOT PROVIDED

- ABI 3730xl or 3500xL Genetic Analyzer with capillary array
- BigDye Terminator v3.1 Cycle Sequencing Kit or compatible dye terminators
- Template DNA (plasmid, PCR product, or BAC DNA)
- Sequencing primers (10-25 nucleotides, 40-60% GC content)
- Thermal cycler with 96-well or 384-well block
- Nuclease-free water or TE buffer (pH 8.0)
- Post-reaction cleanup system (ethanol precipitation, magnetic beads, or gel filtration columns)
- Formamide or Hi-Di Formamide for sample resuspension

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	Clear colorless solution
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 REACTION PROTOCOL USING 5× SEQUENCING BUFFER

This protocol describes the standard cycle sequencing reaction setup using Enzovera 5× Sequencing Buffer with fluorescent dye terminator chemistry for automated Sanger sequencing on capillary electrophoresis instruments.

MATERIALS REQUIRED

- Enzovera 5× Sequencing Buffer
- Fluorescent dye terminator sequencing mix (BigDye-format compatible)
- Purified DNA template (PCR product, plasmid, or BAC DNA)
- Sequencing primer (3.2 pmol per reaction recommended)
- Nuclease-free water
- Thermal cycler with heated lid capability
- 0.2 mL thin-wall PCR tubes or 96-well plate
- Centrifuge for pulse spinning

TEMPLATE REQUIREMENTS

PCR products: 1-10 ng per 100 bp of product length

Plasmid DNA: 200-500 ng double-stranded

BAC DNA: 0.5-1.0 µg

Single-stranded DNA: 50-100 ng

REACTION SETUP

1. Thaw the 5× Sequencing Buffer, dye terminator mix, and primer on ice. Vortex each component briefly and pulse spin to collect contents at the bottom of the tube.

2. Calculate the total number of reactions needed and prepare a master mix containing all components except the DNA template. Include 10% excess volume to account for pipetting loss.

3. Add the following components to a 0.2 mL PCR tube or 96-well plate in the order listed for each 20 µL reaction:

- 4.0 µL of 5× Sequencing Buffer (final concentration 1×)
- 2.0 µL of dye terminator sequencing mix
- 1.0 µL of sequencing primer at 3.2 µM
- X µL of purified DNA template (see template requirements above)
- Nuclease-free water to 20 µL final volume

4. Mix the reaction gently by pipetting up and down 5 times or by brief vortexing. Avoid introducing air bubbles.

5. Pulse spin the tubes or plate for 3-5 seconds to collect all liquid at the bottom and eliminate air bubbles.

6. Place the reaction tubes or plate in a thermal cycler with the heated lid set to 105°C to prevent evaporation.

THERMAL CYCLING CONDITIONS

7. Program the thermal cycler with the following cycling parameters:

Initial denaturation: 96°C for 1 minute (1 cycle)

Followed by 25-35 cycles of:

- Denaturation: 96°C for 10 seconds
- Annealing: 50°C for 5 seconds
- Extension: 60°C for 4 minutes

8. Use 25 cycles for templates less than 500 bp in length or high-quality plasmid DNA.

9. Use 30-35 cycles for templates greater than 500 bp, PCR products, or lower quality DNA preparations.

10. After cycling is complete, hold the reactions at 4°C or proceed immediately to post-reaction cleanup.

POST-REACTION CLEANUP

11. Remove unincorporated dye terminators and reaction components using one of the following methods before capillary electrophoresis:

- Ethanol/sodium acetate precipitation
- Magnetic bead purification
- Spin column purification
- Enzymatic cleanup with exonuclease I and shrimp alkaline phosphatase

12. Store cleaned sequencing reactions at -20°C protected from light if not loading immediately onto the sequencing instrument.

OPTIMIZATION GUIDELINES

13. If sequencing signal is weak, increase the number of thermal cycles by increments of 5 cycles up to a maximum of 40 cycles, or increase template concentration.

14. If high background or dye blob artifacts appear, decrease the number of cycles, reduce template concentration, or ensure thorough removal of primer dimers and contaminants from the template DNA.

15. If peaks are uneven or compressed, verify that the 5× Sequencing Buffer is at 1× final concentration and that the annealing temperature is appropriate for the primer melting temperature (typically 5°C below primer Tm).

16. For GC-rich templates showing compression or secondary structure, add DMSO to a final concentration of 5% (v/v) or betaine to 1.0 M final concentration. Reduce the extension time to 2 minutes if adding organic solvents.

17. For long read lengths beyond 800 bp, increase the extension time to 5-6 minutes and use 30-35 cycles with high-quality template DNA.

QUALITY CONTROL

18. Before sequencing unknown samples, verify thermal cycler performance using a known template and primer set to confirm proper buffer function and cycling conditions.

19. Store the 5× Sequencing Buffer at -20°C when not in use. Avoid repeated freeze-thaw cycles by preparing working aliquots.

20. For best results, use the 5× Sequencing

8. EXPECTED RESULTS

When used at 1× final concentration with Enzovera Sanger sequencing terminator mixes, this buffer produces balanced peak heights with signal-to-noise ratios typically exceeding 20:1 in the first 500 bases. Sequencing reactions yield clean, interpretable traces with average read lengths of 650-850 bases at QV20 when analyzed on capillary electrophoresis systems (ABI 3730xl or equivalent). The buffer maintains enzymatic activity and dye terminator stability throughout thermal cycling, resulting in uniform peak spacing and minimal dye blob artifacts.

9. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommended Action
Poor quality sequencing reads with high background noise	Buffer degradation due to repeated freeze-thaw cycles or improper storage above -20°C	Prepare working aliquots to minimize freeze-thaw cycles. Store buffer at -20°C and verify pH is 9.0 ± 0.1 before use. Replace buffer if stored at room temperature for >2 hours.
Short read lengths (<500 bp) with premature signal decay	Insufficient buffer concentration causing suboptimal PCR conditions or incorrect dilution from 5× to 1× working concentration	Verify correct dilution factor (200 µL buffer + 800 µL nuclease-free water for 1 mL at 1×). Check magnesium concentration is 1.5-2.0 mM in final reaction. Do not over-dilute buffer.
Inconsistent sequencing results between runs	Buffer precipitation due to incomplete thawing or mixing, or contamination with nucleases	Thaw buffer completely at room temperature and vortex thoroughly before use. Centrifuge briefly to collect contents. Use nuclease-free tubes and filtered pipette tips. Discard buffer if precipitation or cloudiness persists after mixing.
Dye blob artifacts or elevated noise in first 50 bases	Incompatible buffer pH affecting BigDye Terminator v3.1 chemistry or presence of residual salts	Confirm buffer pH is 9.0 ± 0.1. Ensure proper cleanup post-cycle sequencing using Enzovera CleanSeq beads or ethanol/sodium acetate precipitation. Increase wash volume during cleanup to remove unincorporated dye terminators.
No sequencing signal or failed capillary electrophoresis run	Buffer expired (>12 months from manufacturing date) or incorrect buffer volume used in cycle sequencing reaction	Check expiration date on buffer label. Use 4 µL of 5× buffer per 20 µL cycle sequencing reaction. Verify BigDye Terminator concentration is 0.25-0.5 µL per reaction. For persistent failures, contact Enzovera Technical

		Support at techsupport@enzovera.com.
Weak signal intensity with acceptable read quality	Suboptimal template concentration or inhibition from residual template purification reagents	Optimize template input: 200-500 ng for PCR products, 500-1000 ng for plasmid DNA. Ensure template is purified and free from EDTA, SDS, or phenol. Increase cycle number to 30-35 cycles if signal remains weak.

10. DOCUMENT CONTROL

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