HI-DI FORMAMIDE (25ML)

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-011
Product Name	Hi-Di Formamide (25ml)
Category	CE Sample Preparation
Pack Size	25ml/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

Enzovera Hi-Di Formamide is a high-purity, deionized formamide intended for resuspension of purified DNA sequencing products prior to capillary electrophoresis analysis on Applied Biosystems genetic analyzers. This reagent denatures DNA secondary structures to enable optimal fragment separation and fluorescence detection during Sanger sequencing runs. Hi-Di Formamide is compatible with BigDye Terminator chemistry and meets performance specifications equivalent to Applied Biosystems Hi-Di Formamide. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Hi-Di Formamide, deionized	1 × 25 mL bottle	2-8°C, protected from light
Product Insert	1 document	Room temperature
Certificate of Analysis	1 document (lot-specific)	Room temperature
Quality Control Data Sheet	1 document	Room temperature

4. MATERIALS REQUIRED BUT NOT PROVIDED

- ABI 3730xl or compatible capillary electrophoresis instrument
- Sanger sequencing reaction products (BigDye Terminator v3.1 or compatible)
- PCR purification or cleanup reagents (SAP/ExoI or magnetic bead-based)
- 96-well optical plates or 8-strip PCR tubes compatible with thermal cycler
- Thermal cycler or heating block capable of 95°C ± 2°C
- Calibrated pipettes (0.5-10 µL and 10-200 µL range) with barrier tips
- Vortex mixer and microcentrifuge (minimum 2000 × g)
- Ice bucket or cooling block for sample preparation

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	Clear colorless liquid

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

HI-DI FORMAMIDE SAMPLE PREPARATION PROTOCOL

Product: Hi-Di Formamide (25 mL)

Application: Capillary Electrophoresis Sample Preparation for Sanger Sequencing

Format: For Research Use Only

PROTOCOL: SAMPLE PREPARATION FOR CAPILLARY ELECTROPHORESIS

MATERIALS REQUIRED

- Enzovera Hi-Di Formamide (Cat. No. contact Technical Support)

- Purified cycle sequencing products (post-cleanup)

- 0.2 mL thin-wall PCR tubes or 96-well optical plates

- Microcentrifuge

- Heat block or thermal cycler

- Pipettes (0.5-20 µL range)

- ABI 3730xl, 3500, 3130xl, or compatible capillary electrophoresis instrument

SAMPLE PREPARATION PROCEDURE

1. Remove Enzovera Hi-Di Formamide from refrigerated storage and allow to equilibrate to room temperature (20-25°C) for at least 15 minutes before use. Invert bottle 3-5 times to ensure complete mixing.

2. Verify that cycle sequencing products have been purified using appropriate cleanup methods (ethanol precipitation, magnetic bead purification, or column purification) to remove unincorporated dye terminators, salts, and reaction buffer components that interfere with electrokinetic injection.

3. Resuspend each dried sequencing pellet in 10 µL of Hi-Di Formamide. For samples in solution, add 10 µL Hi-Di Formamide directly to the purified product. Final volumes may be adjusted based on expected signal strength: use 10-15 µL for standard samples or 7-10 µL for high-concentration templates.

4. Add 0.2 µL of appropriate size standard to each sample. Use GeneScan 500 LIZ, GeneScan 600 LIZ, or MapMarker 1000 ROX depending on expected fragment size range and instrument configuration. Mix size standard with Hi-Di Formamide before adding to samples if processing multiple reactions.

5. Cap tubes or seal plates securely to prevent evaporation during denaturation step.

6. Vortex samples briefly (3-5 seconds) or pipette up and down 5-8 times to ensure complete resuspension of DNA pellet in formamide. Visually confirm no pellet material remains adhered to tube wall or bottom.

7. Centrifuge samples briefly (3-5 seconds at 1000 x g) to collect contents at bottom of tube or plate wells and eliminate air bubbles that interfere with autosampler injection.

8. Denature samples by heating at 95°C for 3 minutes in a thermal cycler or heat block. This step disrupts secondary structure and ensures single-stranded DNA conformation required for optimal separation during capillary electrophoresis.

9. Immediately transfer samples to ice or a cooling block and incubate for 5 minutes. Rapid cooling prevents DNA re-annealing and maintains denatured state. Alternatively, snap-cool samples by placing directly at 4°C for 2 minutes.

10. Centrifuge samples again briefly (3-5 seconds at 1000 x g) to collect condensation and ensure all liquid is at tube or well bottom before loading into capillary electrophoresis instrument autosampler.

11. Load prepared samples into instrument autosampler within 2 hours of denaturation for optimal results. If analysis cannot be performed immediately, store denatured samples at 4°C protected from light for up to 24 hours. Re-denature by heating at 95°C for 2 minutes before analysis if stored longer than 4 hours.

12. Configure capillary electrophoresis run parameters according to instrument specifications: injection voltage 1.2-2.0 kV for 5-30 seconds; run voltage 8.5-15 kV; run temperature 60°C; run time 30-180 minutes depending on polymer type and desired read length.

IMPORTANT NOTES

- Hi-Di Formamide is hygroscopic and will absorb moisture from air, reducing denaturation efficiency. Keep bottle tightly capped when not in use and minimize air exposure during aliquoting.

- Do not autoclave or heat formamide above 100°C as this causes degradation and formation of ammonium formate ions that alter conductivity and reduce injection efficiency.

- Formamide volume-to-sample ratios can be optimized based on DNA concentration. High-concentration samples may require dilution (12-15 µL formamide) to prevent overloading capillary and causing off-scale peaks or dye blob artifacts.

- Use aerosol-barrier pipette tips when handling formamide to prevent cross-contamination between samples.

- Ensure complete removal of ethanol or wash buffer residues from cleanup procedure as these interfere with electrokinetic injection and cause baseline noise.

- For 96-well plate format, use optical or semi-skirted plates compatible with instrument autosampler. Verify plate septa are properly seated to prevent evaporation and cross-contamination.

8. EXPECTED RESULTS

Upon resuspension of purified Sanger sequencing reactions in Hi-Di Formamide, complete denaturation of DNA products should occur, eliminating secondary structure that may cause peak splitting or compression artifacts during capillary electrophoresis. Sequencing samples should exhibit sharp, well-resolved peaks with baseline separation, uniform peak morphology across all four dye channels, and read lengths of 650-850 bases (depending on chemistry and instrument) when analyzed on ABI 3730xl or equivalent genetic analyzers. Background fluorescence should remain low with minimal dye blob interference in the first 15 bases of data.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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