

# 10× RUNNING BUFFER WITH EDTA (500ML)

## INSTRUCTIONS FOR USE

### 1. PRODUCT INFORMATION

<b>Catalog Number</b>	EV-SGR-006
<b>Product Name</b>	10× Running Buffer with EDTA (500ml)
<b>Category</b>	CE Running Buffer
<b>Pack Size</b>	500ml/bottle
<b>Regulatory Status</b>	For Research Use Only (RUO)
<b>OEM Reference</b>	Contact techsupport@enzovera.com
<b>Version</b>	1.0
<b>Issue Date</b>	2026-05-07

### 2. INTENDED USE

This product is a 10× concentrated electrophoresis running buffer containing EDTA, designed for capillary electrophoresis separation on ABI Genetic Analyzers including 3730xl, 3500, and SeqStudio platforms. When diluted to 1× working concentration, this buffer maintains optimal ionic strength and pH conditions for high-resolution separation of fluorescently-labeled DNA fragments during Sanger sequencing and fragment analysis applications. The EDTA component chelates divalent cations to prevent nuclease activity and ensure consistent electrokinetic injection and separation performance. For Research Use Only. Not for use in diagnostic procedures.

### 3. KIT COMPONENTS

Component	Quantity / Volume	Storage
10× Running Buffer with EDTA	500 mL	Room temperature (15-25°C)
Instructions for Use (IFU)	1 document	Room temperature
Certificate of Analysis (CoA)	1 document	Room temperature
Quality Control Certificate	1 document	Room temperature

### 4. MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water (ultrapure, 18.2 MΩ·cm recommended)
- Tris base (molecular biology grade)
- TAPS (N-Tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid)
- EDTA disodium salt dihydrate
- Analytical balance (0.001g precision minimum)
- Magnetic stirrer with stir bar
- pH meter calibrated with pH 7 and pH 10 buffers
- 500 mL volumetric flask or graduated cylinder

### 5. STORAGE AND STABILITY

<b>Storage Temperature</b>	-20°C, protect from light
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<b>Appearance</b>	Clear colorless solution
<b>Shelf Life</b>	12 months from manufacture date
<b>Shipping Conditions</b>	On dry ice
<b>Freeze-Thaw Cycles</b>	Maximum 3 cycles recommended
<b>Working Solution</b>	Stable on ice for up to 8 hours

## 6. PRECAUTIONS AND WARNINGS

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- 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

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### PREPARATION PROTOCOL FOR 10× RUNNING BUFFER WITH EDTA (500 mL)

#### MATERIALS REQUIRED

##### Reagents:

1. Tris base (molecular biology grade)
2. Boric acid (molecular biology grade)
3. EDTA disodium salt dihydrate (molecular biology grade)
4. Ultrapure water (18.2 MΩ·cm resistivity, nuclease-free)

##### Equipment:

1. 1000 mL glass beaker
2. Magnetic stir plate with stir bar
3. Analytical balance (0.01 g precision)
4. pH meter with calibrated electrode
5. 500 mL volumetric flask
6. 0.22 μm sterile filter unit (500 mL capacity)
7. Sterile 500 mL storage bottle
8. Graduated cylinder (100 mL)

#### SAFETY PRECAUTIONS

1. Wear appropriate personal protective equipment including safety glasses, laboratory coat, and nitrile gloves.
2. Work in a well-ventilated area or fume hood when handling powdered reagents.
3. Boric acid is toxic if ingested; handle with care and avoid generating dust.
4. EDTA is a chelating agent; avoid skin contact with concentrated solutions.

#### BUFFER PREPARATION PROCEDURE

##### Step 1. Prepare Workspace

Clean and dry all glassware. Rinse volumetric flask and beaker three times with ultrapure water. Ensure magnetic stir bar is clean and free from contamination.

#### Step 2. Measure Tris Base

Using the analytical balance, accurately weigh 108.0 g of Tris base. Transfer quantitatively to the 1000 mL glass beaker. Record the actual weight measured.

#### Step 3. Measure Boric Acid

Accurately weigh 55.0 g of boric acid on the analytical balance. Add to the beaker containing Tris base. Record the actual weight measured.

#### Step 4. Measure EDTA

Accurately weigh 7.44 g of EDTA disodium salt dihydrate (Na<sub>2</sub>EDTA·2H<sub>2</sub>O) on the analytical balance. Add to the beaker with the other reagents. Record the actual weight measured. This provides 20 mM EDTA in the 10× concentrate.

#### Step 5. Initial Dissolution

Add approximately 300 mL of ultrapure water to the beaker. Place the beaker on the magnetic stir plate and insert the stir bar.

#### Step 6. Dissolve Reagents

Set the stir plate to 400-500 rpm at room temperature (20-25°C). Stir continuously until all solids are completely dissolved. This typically requires 30-45 minutes. The solution will be clear with no visible particles when dissolution is complete.

#### Step 7. Cool Solution

If the dissolution process generated heat, allow the solution to cool to room temperature (20-25°C) before proceeding. This typically requires 15-20 minutes with continued stirring.

#### Step 8. pH Verification

Using the calibrated pH meter, check the pH of the 10× concentrate. The expected pH range is 8.0-8.5 at 25°C. Do not adjust pH as this is a buffered system. If pH falls outside this range, verify reagent quality and measurements.

#### Step 9. Transfer to Volumetric Flask

Quantitatively transfer the dissolved buffer solution to the 500 mL volumetric flask using a funnel if necessary. Rinse the beaker three times with small volumes (10-15 mL each) of ultrapure water and add rinsings to the volumetric flask.

#### Step 10. Bring to Final Volume

Add ultrapure water to the volumetric flask until the bottom of the meniscus reaches the 500 mL calibration mark. Mix thoroughly by inverting the stoppered flask 20-25 times.

#### Step 11. Sterile Filtration

Assemble the 0.22 µm sterile filter unit according to manufacturer instructions. Filter the entire 500 mL of 10× buffer through the sterile filter into the sterile 500 mL storage bottle. Apply gentle positive pressure or vacuum as appropriate for the filter unit design.

#### Step 12. Label and Store

Label the storage bottle with the following information: Product name (10× Running Buffer with EDTA), concentration (10×), volume (500 mL), preparation date, operator initials, lot number (if applicable), and expiration date (12 months from preparation). Store at room temperature (15-25°C) protected from light.

### QUALITY CONTROL CHECKS

1. Visual Inspection: Solution should be clear and colorless with no particulate matter or turbidity.

2. pH Verification: Dilute 10 mL of 10× buffer with 90 mL ultrapure water to make 1× working buffer. Measure pH at 25°C. Expected pH of 1× buffer is 8.3 ± 0.1.

3. Conductivity Test: Measure conductivity of 1× diluted buffer at 25°C. Expected range is 2000-2500

## 8. EXPECTED RESULTS

When diluted to 1× working concentration, this running buffer provides optimal ionic strength and pH conditions for high-resolution capillary electrophoresis separation on ABI 3730xl, 3500, and other genetic analyzers. Users should observe consistent electroosmotic flow, sharp peak resolution, and baseline separation of DNA fragments across the typical read length range (up to 1000 bases for Sanger sequencing applications). EDTA chelation of divalent cations prevents nuclease activity and maintains sample integrity throughout the electrophoresis run.

## 9. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommended Action
High baseline noise or erratic signal in electropherogram	Buffer contamination, degradation, or incorrect dilution ratio	Prepare fresh 1× working solution from unopened 10× stock. Ensure deionized water quality ( $\geq 18 \text{ M}\Omega\cdot\text{cm}$ ). Discard working buffer after 5 days or if cloudiness appears. Filter buffer through 0.2 $\mu\text{m}$ membrane if particulates are visible.
Short read lengths or premature signal dropout	EDTA concentration too high due to dilution error, or buffer pH drift	Verify 10-fold dilution (50 ml stock + 450 ml water). Check pH of working buffer is $8.0 \pm 0.2$ . Replace buffer in capillary array. If pH has shifted, prepare fresh working solution.
Inconsistent migration times between runs	Temperature fluctuations, incomplete buffer exchange, or air bubbles in capillaries	Equilibrate buffer to instrument operating temperature (50-60°C) for 30 minutes before use. Perform full buffer flush cycle. Check for air bubbles in buffer reservoirs and capillary connections. Replace both anode and cathode buffers simultaneously.
Increased dye blob artifacts or spectral overlap	Excessive EDTA chelating residual dye terminators, or buffer ionic strength imbalance	Ensure ExciS-Seq purification was performed correctly on samples. Verify 10× concentrate was mixed thoroughly before dilution. Check capillary array conditioning; run a blank injection to flush system. Replace polymer if issue persists.
Loss of resolution in homopolymer regions	Degraded EDTA reducing metal chelation efficiency, affecting DNA mobility	Check expiration date of 10× stock. Store at room temperature (15-25°C) and protect from light. Avoid repeated freeze-thaw if stored at 4°C. Prepare fresh 1× working buffer every 3-5 days. Verify capillary array is clean and polymer is fresh.

No signal or very weak fluorescence detected	Dilution error resulting in insufficient ionic strength, or buffer/polymer incompatibility	Confirm correct 1:10 dilution ratio. Ensure buffer is compatible with POP-7 or POP-6 polymer being used. Check that sample cleanup removed excess salts but retained DNA. Verify instrument laser and detector function with QC standards. Replace both buffer and polymer if necessary.
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## 10. DOCUMENT CONTROL

<b>Document Number</b>	IFU-EV-SGR-006
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