# HP HAIRPIN REAGENT (7-DEAZA DATP, 1ML)

INSTRUCTIONS FOR USE

## 1. PRODUCT INFORMATION

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| **Catalog Number** | EV-SGR-010 |
| **Product Name** | HP Hairpin Reagent (7-deaza dATP, 1ml) |
| **Category** | Sanger Sequencing Additive |
| **Pack Size** | 1ml/tube |
| **Regulatory Status** | For Research Use Only (RUO) |
| **OEM Reference** | Contact techsupport@enzovera.com |
| **Version** | 1.0 |
| **Issue Date** | 2026-05-07 |

## 2. INTENDED USE

The HP Hairpin Reagent is a 7-deaza-2′-deoxyadenosine triphosphate (7-deaza dATP) additive designed to improve Sanger sequencing read quality through GC-rich regions, secondary structures, and homopolymeric adenine runs by substituting for standard dATP in cycle sequencing reactions. This reagent destabilizes DNA hairpin formation during thermal cycling, reducing premature termination events and compression artifacts that cause ambiguous base calls in difficult templates. The HP Hairpin Reagent is compatible with BigDye chemistry and capillary electrophoresis instruments including ABI 3730xl, 3500, and SeqStudio systems. For Research Use Only. Not for use in diagnostic procedures.

## 3. KIT COMPONENTS

| Component | Quantity / Volume | Storage |
|---|---|---|
| 7-deaza-dATP Hairpin Reagent (40 mM) | 1 × 1 mL | -20°C |
| BigDye Terminator v3.1 Sequencing Buffer (5X) | 1 × 2 mL | -20°C |
| Hairpin Reagent Protocol Guide | 1 guide | Room temperature |
| Quality Control Certificate | 1 certificate | Room temperature |

## 4. MATERIALS REQUIRED BUT NOT PROVIDED

- Sanger sequencing kit (BigDye Terminator v3.1 or compatible chemistry)
- Template DNA (plasmid, PCR product, or BAC clone)
- Sequencing primers (10 µM stock concentration)
- 5x sequencing buffer or reaction buffer
- Thermal cycler with cycle sequencing capability
- Ethanol precipitation reagents (3M sodium acetate, 95% ethanol)
- Capillary electrophoresis instrument (ABI 3730xl, 3500, or equivalent)
- Hi-Di formamide or equivalent polymer loading solution

## 5. STORAGE AND STABILITY

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| **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

HP HAIRPIN REAGENT PROTOCOL

7-deaza-2'-deoxyadenosine-5'-triphosphate (7-deaza dATP)

For Research Use Only

PRODUCT OVERVIEW

HP Hairpin Reagent contains 7-deaza-2'-deoxyadenosine triphosphate (7-deaza dATP), a modified nucleotide analog that replaces standard dATP in Sanger cycle sequencing reactions. The 7-deaza modification at the N7 position eliminates the hydrogen bonding site, thereby destabilizing secondary structures including GC-rich hairpins, stem-loops, and compression artifacts that cause premature termination and signal dropout in standard sequencing reactions.

APPLICATIONS

- Resolution of GC-rich templates (>65% GC content)

- Sequencing through hairpin structures and inverted repeats

- Reading through homopolymeric A/T runs longer than 8 nucleotides

- Elimination of compression bands in difficult templates

- Improved read length through structured regions

- Recovery of sequence data from templates with strong secondary structure

PROTOCOL: CYCLE SEQUENCING WITH HP HAIRPIN REAGENT

MATERIALS REQUIRED

- HP Hairpin Reagent (7-deaza dATP), 10 mM stock concentration

- BigDye Terminator v3.1 Cycle Sequencing Kit or compatible reagent

- Purified DNA template (plasmid, PCR product, or BAC DNA)

- Sequencing primer, 3.2 pmol/µL stock concentration

- 5X Sequencing Buffer (supplied with BigDye kit or equivalent)

- Thermal cycler with heated lid and 0.1°C temperature control

- Nuclease-free water

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

TEMPLATE PREPARATION

1. Quantify template DNA using spectrophotometry at A260/A280 or fluorometric quantitation (Qubit, PicoGreen).

2. Dilute template to appropriate concentration in nuclease-free water:

- Plasmid DNA: 150-300 ng per reaction

- PCR products (100-500 bp): 5-10 ng per reaction

- PCR products (500-1000 bp): 10-20 ng per reaction

- PCR products (1000-2000 bp): 20-40 ng per reaction

- BAC DNA: 0.5-1.0 µg per reaction

3. Verify template purity. A260/A280 ratio should be 1.8-2.0. Samples with ratios below 1.7 or above 2.1 may require cleanup using silica column purification or ethanol precipitation.

REACTION SETUP

4. Thaw HP Hairpin Reagent on ice. Mix gently by pipetting. Do not vortex. Centrifuge briefly to collect liquid.

5. Thaw all sequencing reaction components on ice, including BigDye Terminator mix, 5X buffer, and primer stock.

6. Prepare master mix on ice in the following order for one reaction (scale proportionally for multiple reactions, prepare 10% excess):

Component Volume per Reaction

5X Sequencing Buffer 2.0 µL

BigDye Terminator v3.1 Ready Mix 1.0 µL

HP Hairpin Reagent (10 mM 7-deaza dATP) 1.0 µL

Sequencing primer (3.2 pmol/µL) 1.0 µL

Nuclease-free water Variable to 10 µL

Template DNA Variable (see step 2)

Final reaction volume: 10 µL

Final 7-deaza dATP concentration: 1.0 mM

7. Mix master mix thoroughly by pipetting up and down 5-6 times. Avoid introducing air bubbles.

8. Aliquot appropriate volume of master mix (template DNA volume subtracted) into 0.2 mL PCR tubes or 96-well plate wells.

9. Add template DNA to each reaction tube. Mix by pipetting 3-4 times.

10. Centrifuge reaction tubes briefly (5 seconds at 1000 x g) to collect liquid at tube bottom and eliminate air bubbles.

11. Cap tubes securely or seal plate with optical adhesive film rated for thermal cycling.

CYCLE SEQUENCING CONDITIONS

12. Place reaction tubes or plate in thermal cycler with heated lid set to 105°C.

13. Program thermal cycler with the following standard cycling protocol:

Initial denaturation: 96°C for 1 minute

25-35 cycles of:

- Denaturation: 96°C for 10 seconds

- Annealing: 50°C for 5 seconds

- Extension: 60°C for 4 minutes

Hold: 4°C indefinitely

14. For extremely GC-rich templates (>70% GC), modify cycling parameters

## 8. EXPECTED RESULTS

When incorporated into Sanger sequencing reactions, HP Hairpin Reagent enables successful read-through of GC-rich hairpins, palindromic sequences, and homopolymeric A/T runs that typically cause premature termination or compression artifacts in standard BigDye chemistry. Users should observe extended read lengths past previously problematic regions (typically 50-150 bases further), elimination of dye blob artifacts at hairpin sites, and uniform peak spacing through homopolymeric runs when 7-deaza dATP replaces 20-50% of standard dATP in the reaction mix. Optimal results require empirical titration for each template type, as excessive substitution may reduce overall signal intensity while insufficient substitution fails to resolve secondary structure.

## 9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

## 10. DOCUMENT CONTROL

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|---|---|
| **Document Number** | IFU-EV-SGR-010 |
| **Version** | 1.0 |
| **Status** | DRAFT — Pending Authorisation |
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| **Approved By** | Pending |
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