INSTRUCTIONS FOR USE

VeraDye™ Terminator v3.1

Cycle Sequencing Kit

Catalog No.: EV-SGR-002

Version: 1.1 | For Research Use Only

Enzovera Life Sciences · 2600 Hilltop Drive, Richmond, CA · orders@enzovera.com · www.enzovera.com

1. Product Information

Product name	VeraDye™ Terminator v3.1 Cycle Sequencing Kit
Catalog number	EV-SGR-002
Intended use	Sanger cycle sequencing of single- or double-stranded DNA templates
Storage conditions	−20°C · Protected from light · Avoid repeated freeze-thaw cycles (max 10 cycles)
Regulatory status	For Research Use Only (RUO). Not for diagnostic or therapeutic use.

2. Product Description

The VeraDye™ Terminator v3.1 Cycle Sequencing Kit is a complete, ready-to-use cycle sequencing reagent system based on the Sanger chain termination method with capillary electrophoresis detection. The kit is supplied as a 2.5× concentrated Sequencing Premix, fully optimized to provide robust and flexible chemistry for all standard Sanger sequencing applications including de novo sequencing and resequencing.

VeraDye™ is formulated for fluorescence-based cycle sequencing on single-stranded or double-stranded DNA templates, including PCR amplicons and plasmid DNA. The v3.1 formulation generates sequencing data with uniform peak heights, optimized signal balance, long high-quality reads, and highly accurate base assignments.

VeraDye™ Terminator v3.1 Cycle Sequencing Kits are a direct drop-in replacement for BigDye® Terminator v3.1 Cycle Sequencing Kits. No changes to existing protocols, reaction volumes, or instrument settings are required. VeraDye™ is compatible with all standard Sanger sequencing workflow products and all major capillary electrophoresis platforms from Applied Biosystems®.

3. General Usage Guidelines

- Avoid excess freeze-thaw cycles. Limit to no more than 10 cycles per reagent tube. If extended storage with frequent access is required, aliquot reagents into smaller single-use volumes.
- Before each use, allow frozen reagents to thaw on ice or at room temperature. Do not use heat to thaw.
- Keep thawed reagents on ice during use. Do not leave reagents at room temperature for extended periods.
- Protect dyes from light at all times to prevent photobleaching and signal loss.

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 all reagents and buffers in this kit. If contact with skin or eyes occurs, wash immediately with large amounts of water and seek medical attention if irritation persists. Dispose of reagents in accordance with applicable local, state, and federal regulations.

5. Protocol

The VeraDye™ cycle sequencing workflow comprises four steps: (1) prepare DNA templates, (2) perform cycle sequencing, (3) purify cycle sequencing products, and (4) perform capillary electrophoresis.

5.1 Template Preparation

For optimal results, purify PCR products prior to cycle sequencing to remove residual dNTPs and primers. Acceptable purification methods include silica-membrane spin columns, magnetic bead-based PCR cleanup (equivalent to AMPure XP), and enzymatic cleanup (exonuclease I / shrimp alkaline phosphatase). The sequencing template should be as free as possible from proteins, RNA, chromosomal DNA, PCR primers, residual dNTPs, enzymes, buffer salts, organic chemicals, and detergents, all of which can interfere with the sequencing reaction.

Use the following guidelines for template input quantity per sequencing reaction:

DNA Template Type	Recommended Input Quantity
PCR product · 100–200 bp	3–10 ng
PCR product · 500–1,000 bp	5–20 ng
PCR product · 1,000–2,000 bp	10–40 ng
PCR product · >2,000 bp	20–50 ng
Plasmid DNA (miniprep/maxiprep)	150–300 ng
Bacterial genomic DNA	2–3 µg

Too little template results in weak signals and elevated background noise. Excess template may cause signal overload and shortened readable read length. Optimize template input within these ranges for best results.

5.2 Sequencing Primer Guidelines

Use high-quality, HPLC- or desalt-purified sequencing primers. The most common primer artifact is the N-1 stutter, caused by incompletely synthesized primer molecules. Store sequencing primers at a concentration of 3.2–5.0 µM (µmol/L) at −20°C. Avoid excess freeze-thaw cycles. Use 3–5 pmol of primer per sequencing reaction.

5.3 Diluting and Reaction Setup

The VeraDye™ Sequencing Premix is supplied at 2.5× concentration. Reactions may be diluted using the supplied 5× Sequencing Buffer to extend the reagent and optimize signal levels. Ensure the final reaction concentration is 1×. The intrinsic buffer concentration within the premix is 2.5×; a standard undiluted reaction uses 8 µL premix in a total volume of 20 µL.

Use the following formula to calculate the volume of 5× Sequencing Buffer required when diluting the premix:

$$V_B = (V^T / 2.5 - V_m) / 2$$

V_B = volume of 5× Sequencing Buffer · V^T = total reaction volume · V_m = volume of VeraDye™ Premix

Example reaction setup (20 µL total volume):

Reagent	Volume
VeraDye™ 2.5× Sequencing Premix	2.0 µL
5× Sequencing Buffer (EV-SGR-003)	3.0 µL
DNA Template	2.0 µL
Sequencing Primer (3.2–5.0 µM)	2.0 µL
Nuclease-free water	11.0 µL
Total volume	**20.0 µL**

5.4 Thermal Cycling Protocol

Use a calibrated thermal cycler (96-well, 0.2 mL standard format) with a heated lid set to 105°C and a thermal ramp of approximately 1°C/sec.

Set the reaction volume in the thermal cycler software to 20 µL.

Step	Temperature	Duration
Initial denaturation	96°C	45 sec
Denature (35 cycles)	96°C	10 sec
Anneal (35 cycles)	50°C	5 sec
Extend (35 cycles)	60°C	4 min
Hold	4°C	∞

5.5 Purification of Cycle Sequencing Products

Prior to capillary electrophoresis, purify the cycle sequencing reaction products to remove unincorporated dye-labeled ddNTPs (dye terminators) and residual salts. Failure to adequately purify

the products results in dye blob artifacts in the electropherogram that obscure bases in the early read region and reduce usable read length.

Recommended cleanup methods compatible with VeraDye™:

- Magnetic bead-based dye terminator removal (EV-SGR-007, Sequencing Product Purification Magnetic Beads · equivalent to CleanSEQ)
- Ion-exchange resin spin column cleanup (EV-SGR-008, Sequencing Cleanup Resin 50 mL)
- Alternative: BigDye Xterminator™ equivalent resin cleanup systems

6. Capillary Electrophoresis Instrument Compatibility

VeraDye™ purified extension products are compatible with the following capillary electrophoresis platforms:

- Applied Biosystems® 310 DNA Sequencer
- Applied Biosystems® 3100 (Avant) Genetic Analyzer
- Applied Biosystems® 3130 / 3130xl Genetic Analyzer
- Applied Biosystems® 3500 / 3500xL Genetic Analyzer
- Applied Biosystems® 3730 / 3730xl DNA Analyzer
- Applied Biosystems® SeqStudio™ and SeqStudio™ Flex Genetic Analyzer

Use POP-7 polymer (EV-SGR-004) on ABI 3730/3730xl and 3500 series instruments. Use POP-4 polymer (EV-SGR-005) on ABI 3100 and 3130 series instruments.

7. Dye Set / Matrix File / Spectral Calibration

VeraDye™ Terminator v3.1 Cycle Sequencing Kits are optimized to run with Dye Set / Filter Set Z for BigDye® Terminator v3.1. Refer to your instrument user manual for instructions on spectral calibration using this dye set. Calibration can be performed using the pGEM Control DNA and −21 M13 Primer included in the kit.

8. Data Analysis

For primary base calling, use the sequencing analysis software provided with your genetic analyzer. Use the KB Base Caller in combination with a DyeSet/Primer file compatible with BigDye® v3.1. No modification to base-calling settings or dye matrix files is required when transitioning from BigDye® v3.1 to VeraDye™ v3.1.

9. Controls

Each VeraDye™ Terminator v3.1 Cycle Sequencing Kit includes pGEM Control DNA and −21 M13 Primer for workflow validation and troubleshooting. Use 1 µL of the pGEM Control and 1 µL of the −21 M13 Primer in a standard cycle sequencing reaction to verify reagent performance and instrument function independent of the user's template and primer variables.

If the control reaction succeeds but the sample reactions fail, investigate template quality, purity, and quantity, or primer design and concentration. If both control and sample reactions fail, contact Enzovera technical support.

10. Troubleshooting

Observation	Possible Cause	Corrective Action
Weak or absent signal	Insufficient template quantity	Increase template input within recommended range
	Template impurity / contaminants	Re-purify template using column cleanup or magnetic beads
	Primer concentration too low	Use 3–5 pmol primer per reaction
Short reads / signal drops	Excess template causing signal overload	Reduce template input
	Inadequate dye terminator cleanup	Ensure complete purification; check bead or resin volume
Dye blob artifacts in early read	Incomplete removal of unincorporated terminators	Extend purification incubation; increase wash stringency
N-1 stutter peaks	Primer contains non-full-length product	Use HPLC-purified or desalt-purified primer; store at −20°C
Poor signal through GC-rich or structured region	Template secondary structure interfering with extension	Add EV-SGR-010 HP Hairpin Reagent (7-deaza dATP) to sequencing reaction
Control reaction fails	Reagent degradation or freeze-thaw damage	Check storage conditions; do not use reagents that have exceeded 10 freeze-thaw cycles

11. Technical Support

For technical assistance with VeraDye™ products, please contact: techsupport@enzovera.com

12. Legal Notice

BigDye is a registered trademark of Thermo Fisher Scientific. Applied Biosystems and SeqStudio are trademarks of Thermo Fisher Scientific. VeraDye is a trademark of Enzovera Life Sciences. All other product names and trademarks are the property of their respective owners. This product is for research use only and is not intended for diagnostic, therapeutic, or clinical use.

Although the information in this document is presented in good faith and believed to be correct at the time of publication, Enzovera Life Sciences makes no representations or warranties as to its completeness or accuracy. Enzovera has no liability for any errors or omissions in this document or for your use of it.

IFU-EV-SGR-002 · Version 1.1 · 2025-04-23