BrilliantDye™Terminator Cycle Sequencing Kit v1.1

User manual

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Product and Company Information

Product name: BrilliantDye™ Terminator Cycle Sequencing Kit v1.1
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Description

The BrilliantDye™ Terminator Cycle Sequencing Kit is a complete kit, based on the trusted Sanger Chain Termination method. The kit is delivered as a 2.5x concentrated ready-reaction premix, fully optimized for a highly flexible chemistry, designed for all kinds of Sequencing applications, including de novo sequencing and resequencing. The kit generates data with uniform peak heights and optimized signal balance to produce long, high-quality reads.

By combining the robustness and better mobility of the dye set, with a novel enzyme and optimized dNTP/ddNTP ratios for longer reads, BrilliantDye is the universal Sequencing kit that can be used for all kinds of applications:

- Sequencing of (short) PCR products with fast, rapid, or ultra-rapid run modules and High Quality Base Calling for the small fragments
- Sequencing of (long range) PCR products with maximum read lengths with standard or long run modules
- Sequencing of Plasmid DNA with maximum read length
- Sensitive Heterozygote detection with optimized peak heights distribution

Kit Content

p/n	Reactions	RR seq. Premix	5 x Seq. Buffer	pGem control	M13(-21) primer
BRD1-010	10	1 x 80 µL	1 x 0.25 mL		
BRD1-024	24	1 x 192 µL	1 x 0.65 mL	1 x 10 µL	1 x 10 µL
BRD1-100	100	1 x 800 µL	1 x 2.0 mL	1 x 10 µL	1 x 10 µL
BRD-1000	1000	10 x 800 µL	8 x 2.0 mL	1 x 50 µL	1 x 50 µL
BRD1-5000	5000	50 x 800 µL	40 x 2.0 mL	5 x 50 µL	5 x 50 µL

Protocol

The BrilliantDye Terminator Cycle Sequencing Kit contain all required reagent components for the sequencing reaction in a ready reaction, pre-mixed format. These reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, including PCR fragments and plasmids.

Diluting: The kit includes BrilliantDye Terminator Sequencing Buffer (5X), which has been optimized for use with the reaction mix. This buffer should be used for any reaction optimization.

Purification of PCR templates: For optimum results, purify the PCR product before sequencing by removing dNTPs and primers. We recommend Nimagen's AmpliClean™ Magnetic bead based PCR Cleanup kit (AP-005, AP-050 or AP-500) or Ex'S-Pure™ enzymatic PCR cleanup kit (EXS-100, EXS-500 or EXS-5000).

Template Quality/Quantity: A common cause of poor Sequencing quality is the quality or the quantity of the template used for the sequencing reaction. The template should be as much as possible free from proteins, RNA, chromosomal DNA, PCR primers, dNTPs, enzymes, buffer components, salts, organic chemicals and residual detergents.

For setting up the cycle sequencing reaction, use the following guidelines in template quantity:

PCR 100–200 bp	2–4 ng
PCR 200–500 bp	5–10 ng
PCR 500–1000 bp	5–20 ng
PCR 1000–2000 bp	10–40 ng
>2000 bp	20–50 ng
Plasmid DNA	150–300 ng

Too low template results in weak signals and elevated signal-to-noise (S/N) ratios. Too much template results in short reads with overloaded signals.

Primer Quality/Quantity: Always use high quality primers for Cycle Sequencing, as well as for generating PCR template. Most common cause of primer issues is the so-called N-1 artifact, caused by primer solutions that contain partially non full-length product, causing the typical "n-1 stutter peaks". We recommend to store Sequencing primers in a concentration of 5 µM (=5 pMol/µL) at -20°C and avoid many freeze-thaw cycles. Use 3-5 pMol sequencing primer per reaction.

Reaction Setup: The 2.5x concentrated BrilliantDye ready-reaction premix can be diluted, using the provided 5x Sequencing Buffer. Always make sure that the end concentration is 1x. Be aware that the Premix has an intrinsic buffer concentration of 2.5x, → a standard reaction should contain 8 µL of the premix in an end volume of 20 µL. However we do not recommend to use full reactions, in order to prevent overloaded signals and to save material. General rule for using the 5x Sequencing Buffer in combination with the 2.5x rr Premix:

µL Buffer = 0.5 * ((**µL total volume** / 2.5) – (**µL Premix**)).

Example:

- 1 µL BrilliantDye rr Premix
- 1.5 µL 5x Sequencing Buffer: *0.5*((10/2.5) – 1) = 0.5*(4-1) = 0.5*3*
- 1 µL Template
- 1 µL primer (5 pMol)
- 5.5 µL Water

10 µL

Thermal Cycling: For the Cycle Sequencing reaction we recommend any brand of High Quality thermal cycler with the following features:

- 96 well (0,2 mL standard format)
- Heated lid (105 °C)
- Thermal ramp of appr. 1°C / sec.
- Fully programmable in multiple stages
- Capability to cool down to 4°C at the end of the program

Example: Veriti® PCR System from Applied Biosystems:

Protocol for Thermal Cycling:

initial denaturation	96°C	60 sec
28 cycles	96°C	10 sec
	50°C	5 sec
	72°C	1 min. 15 sec.
hold	4°C	∞

Purification of the Extension Product: Before capillary electrophoresis the Cycle Sequencing products need to be purified to remove unincorporated fluorescent ddNTPs and salts. Several methods can be used for this purpose, including Ethanol Precipitation, Sephadex based filtration (Edge Biosystems) or magnetic bead based purification. We recommend to use Nimagen's D-Pure™ DyeTerminator Cleanup kit as a cost-effective, high quality purification method, in combination with a Nimagen 12-wells magnet stand (for low-throughput manual cleanup) or a Alpaqua 96-well Ring Magnet (for automated cleanup), also available via Nimagen.

Instrument platforms: The purified extension products can be analyzed by capillary electrophoresis on one of the following platforms:

- Applied Biosystems 310 DNA sequencer
- Applied Biosystems / Hitachi 3100 (Avant) Genetic Analyzer
- Applied Biosystems / Hitachi 3130 (XL) Genetic Analyzer
- Applied Biosystems / Hitachi 3500 (xL) Genetic Analyzer
- Applied Biosystems / Hitachi 3730 (XL) DNA Analyzer

Dye Set / Matrix File / Spectral Calibration: The kit is optimized to run with Filterset E (Dye Set E for BigDyeTerminator v1.1). Refer to your instrument manual how to calibrate the system with this Dye Set. Optionally, Filterset Z can be used, but is slightly sub optimal.

Control provided in the kit: All BrilliantDye kits (except the BRB1-010) contain control DNA template (pGEM plasmid DNA) and control primer (-21M13). Use 1 µL of this template and 1 µL of the primer in a Sequencing Reaction to verify the performance of your total workflow and troubleshoot issues, correlated to your templates and/or primer. The sequence of the first part of the pGEM control:

TGTAAAACGACGGCCAGT (–21 M13 primer) –
GAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTC
TATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGC
CGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAA
ACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGAC
TCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAG
GAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGA
CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG
TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAAC
TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGG
TGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG