Instructions For Use

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BrilliantDye® Terminator v1.1

Cycle Sequencing Kit

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

BrilliantDye™ Terminator v1.1 Cycle Sequencing Kit

REF BRD1-024, BRD1-100, BRD1-1000, BRD1-5000, BRD1-25K

Research Use Only

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Symbols Used on Product Labels and in Instructions For Use

Symbol	Description
	Manufacturer
	Use-by date
	Lot number
	Reference number
	Temperature limit for storage
	Contains sufficient for <*n*> tests
	Matrix code containing the reference number, lot number and use-by date

Product Description

The BrilliantDye™ Terminator v1.1 Cycle Sequencing Kit is a complete kit, based on the trusted Sanger Chain Termination method using capillary electrophoresis. The kit is delivered as a 2.5x concentrated Ready Reaction (RR) Sequencing Premix, fully optimized to provide a robust and highly flexible chemistry, designed for all kinds of DNA sequencing applications, including de novo and resequencing.

BrilliantDye™ is developed for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, including PCR fragments and plasmids. BrilliantDye™ generates data with uniform peak heights and optimized signal balance to produce long, high-quality reads, as well as highly accurate base assignments.

BrilliantDye™ Terminator v1.1 Cycle Sequencing Kits are a drop-in replacement for BigDye® Terminator v1.1 Cycle Sequencing Kits without having to change protocol, volume, or setting. The products can be used with other products in the Sanger sequencing workflow (e.g. NimaPOP™ or POP™ polymers and buffers) on Applied Biosystems® 3130, 3500, 3730 and SeqStudio™ Flex Genetic Analyzers.

Kit Contents and Storage

BrilliantDye™ Terminator v1.1 Cycle Sequencing Kits include all required reagents for sequencing 24, 100, 1000, 5000, or 25000 single-stranded or double-stranded DNA templates.

Each BrilliantDye™ Kit contains 4 reagents: Ready Reaction (RR) Sequencing Premix, 5x Sequencing Buffer, pGEM Control and -21 M13 Primer:

Reference	Reactions	RR Seq. Premix	5x Seq. Buffer	pGEM Control	-21 M13 Primer	Storage
BRD1-024	24	1 x 192 µL	1 x 0.65 mL	10 µL	10 µL	Store kit at -15 °C to -25 °C, protected from light and avoid repeated freeze-thaw cycles.
BRD1-100	100	1 x 800 µL	1 x 2.0 mL	10 µL	10 µL	
BRD1-1000	1000	10 x 800 µL	8 x 2.0 mL	50 µL	50 µL	
BRD1-5000	5000	2 x 20 mL	2 x 28 mL	50 µL	50 µL	
BRD1-25K	25000	10 x 20 mL	10 x 28 mL	50 µL	50 µL	

Contents	Reference BRD1-024	Reference BRD1-100	Reference BRD1-1000	Reference BRD1-5000	Reference BRD1-25K
RR Seq. Premix	BRD1-t024	BRD1-t100	10x BRD1-t100	2x BRD1-t2500	10x BRD1-t2500
5x Seq. Buffer	1x BRB-650	1x BRB-2000	8x BRB-2000	2x BRB-110	10x BRB-110
pGEM Control	1x PGEM-10	1x PGEM-10	1x PGEM-50	1x PGEM-50	1x PGEM-50
-21 M13 Primer	1x M13F-10	1x M13F-10	1x M13F-50	1x M13F-50	1x M13F-50

General Usage Guidelines

- Avoid excess freeze-thaw cycles (no more than 10). If needed, aliquot the reagents into smaller amounts.
- Before each use of the kit, allow the frozen stocks to thaw on ice or at room temperature (do not heat).
- Keep thawed materials on ice during use. Do not leave reagents at room temperature for extended periods.
- Protect dyes from light to avoid photobleaching.

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice and wear protective eyewear, gloves and lab coat when handling the reagents and buffers supplied in this kit. Wash body parts with ample amount of water immediately if they come in contact with the reagents and buffers. Seek medical help if needed.

Protocol

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

Purification of PCR Templates

For optimum results, purify the PCR product before cycle sequencing by removing dNTPs and primers. We recommend NimaGen's AmpliClean™ Magnetic Bead-based PCR Cleanup Kit (AP-005, AP-050, AP-500) or ExS-Pure™ Enzymatic PCR Cleanup Kit (EXS-100, EXS-500, EXS-5000). AmpliClean™ and ExS-Pure™ are the proven equivalents of respective Beckman AMPure XP and Thermo Fisher ExoSAP-IT™ reagents for PCR purification.

Template Quality/Quantity

A common cause of poor sequencing results 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.

The quantity of PCR product is optimized to maximize the number of primer binding sites for the BrilliantDye™ reaction and is dependent upon the length and purity of the PCR product.

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

DNA Template	Quantity
PCR 100–200 bp	3 – 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
Bacterial genomic DNA	2 – 3 µg

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

Primer Quality/Quantity

Always use high quality primers for cycle sequencing, as well as for generating PCR templates. The 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 3.2 - 5 µM (pmol/µL) at -20 °C and avoid excess freeze-thaw cycles. Use 3 - 5 pmol sequencing primer per reaction.

Diluting and Reaction Setup

Some cycle sequence reactions may be optimized using diluted 2.5x BrilliantDye™ RR Sequencing Premix. The 5x Sequencing Buffer has been optimized for use with the RR Premix and should be used for any reaction optimization. When diluting, always make sure that the final end reaction concentration is 1x. NOTE: Premix has an intrinsic buffer concentration of 2.5x, i.e. 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 BrilliantDye™ RR Sequencing Premix:

$$V_B = (V_T/2.5 - V_M)/2$$

V_B = Volume of 5x Sequencing Buffer in the reaction

V_T = Total sequencing reaction volume

V_M = Volume of BrilliantDye v1.1 RR Sequencing Premix in the reaction

Example of reaction setup:

1 µL 2.5x BrilliantDye™ RR Sequencing Premix (V_M)
1.5 µL 5x Sequencing Buffer (V_B)
1 µL DNA Template
1 µL Primer (3.2 - 5 pmol)
5.5 µL H_2O
10 µL (V_T)

Perform Cycle Sequencing

For the cycle sequencing reaction we recommend any brand of high-quality thermal cycler (e.g. Applied Biosystems, Bio-Rad) with the following features:

- 96-well (0.2 mL standard format) or 384-well
- Heated lid (105 °C)
- Thermal ramp of approx. 1 °C / sec
- Ability to cool down to 4 °C at the end of the program

1. Place the tubes or plate(s) in a thermal cycler and set the correct volume:
    a. 20 µL for 96-well reaction plates
    b. 10 µL for 384-well reaction plates
2. Perform cycle sequencing, using the following thermal protocol

Initial denaturation		96 °C	45 sec
25x cycle	Denature	96 °C	10 sec
	Anneal	50 °C	5 sec
	Extend	60 °C	4 min
Hold (until ready to purify)		4 °C	∞

Purification of Cycle Sequencing Products

Prior to capillary electrophoresis, the cycle sequencing products need to be purified, removing unincorporated dye terminators (fluorescent ddNTPs) and salts. We recommend using NimaGen's D-Pure™ Dye Terminator Magnetic Cleanup Kit (DP-005, DP-050, DP-500) as a cost-effective, high-quality cycle sequencing product purification method, in combination with an Alpaqua® 96-well Magnet Plate, also available from NimaGen.

Alternatively, iX-Pure™ Dye Terminator Cleanup Kit (IXP-100, IXP-1000, IXP-2500), the BigDye Xterminator™ equivalent, can be used to effectively remove unincorporated dye terminators, free salts from the post-sequencing reaction and stabilize the post-purification reactions.

Capillary Electrophoresis Instrument Compatibility

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

- Applied Biosystems® 3130/3100XL Genetic Analyzer
- Applied Biosystems® 3500/3500xL Genetic Analyzer
- Applied Biosystems® 3730/3730xl DNA Analyzer
- Applied Biosystems® SeqStudio™ (Flex) Genetic Analyzer

Dye Set / Matrix File / Spectral Calibration

The BrilliantDye™ Terminator v1.1 Cycle Sequencing Kits are optimized to run with Filterset Z for BigDye® Terminator v1.1. Refer to your instrument manual how to calibrate with this Dye Set. Calibration can be performed using the pGEM Control and -21 M13 Primer included in the kit.

Data Analysis

For primary base calling, the easiest option is to use sequencing analysis software provided with the automated sequencer. We recommend to use the KB Base Caller, in combination with a DyeSet/Primer file, suitable for BigDye® v1.1.

For improved basecalling with longer read lengths, NimaGen recommends PeakTrace (https://www.nucleics.com/peaktrace/).

Controls

All BrilliantDye™ Terminator Cycle Sequencing Kits contain pGEM Control and -21 M13 Primer. Use 1 µL of this template and 1 µL of the primer in a cycle 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:

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TGTAAAACGACGGCCAGT (-21 M13 primer) -
GAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCA
TGCAAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAA
TTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGT
GAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTA
ATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCT
GCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGG
GGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGC
GTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
AGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTAC
CGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC
GGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGG
TAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAG
CAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGT
ATTTGGTATCTGCGCTCTGCTGAAG
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Customer Support

For technical assistance, please contact us at techsupport@nimagen.com.

Revision History

Section	Summary of changes	Version	Date
All	Not applicable. New document.	3.1	2018-09-01
All	New layout. New introduction (Product Description). Kit Contents and Storage. General Precautions.	4.0	2023-06-23

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