

BigDye™ Terminator v3.1 Cycle Sequencing Kit

Catalog Numbers 4337454, 4337455, 4337456, 4337457, 4337458

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *BigDye™ Terminator v3.1 Cycle Sequencing Kit User Guide* (Pub. no. 4337035). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users of the BigDye™ Terminator v3.1 Sequencing Standard Kit (Cat. Nos. 4337454, 4337455, 4337456, 4337457, and 4337458). See the *BigDye™ Terminator v3.1 Cycle Sequencing Kit User Guide* (Pub. No. 4337035) for detailed instructions and troubleshooting.

Product description

The BigDye™ Terminator v3.1 Cycle Sequencing Kit provides pre-mixed reagents for Sanger sequencing reactions.

The kit includes BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer, which is specifically optimized for use with the BigDye™ Ready Reaction mixes.

The kit has been formulated to deliver robust performance across a wide variety of DNA sequences while maximizing readlengths. When used in combination with Minor Variant Finder Software, the kit can also be used to detect variants as low as 5% in a sample (see *Minor Variant Finder Software User Guide* (Pub. No. MAN0014835)).

Workflow



Prepare templates

Template quantity

Table 1 Recommended DNA quantities

DNA template	Quantity
PCR product:	
• 100–200 bp	1–3 ng
• 200–500 bp	3–10 ng
• 500–1000 bp	5–20 ng
• 1000–2000 bp	10–40 ng
• >2000 bp	20–50 ng
Single-stranded DNA	25–50 ng
Double-stranded DNA	150–300 ng
Cosmid, BAC	0.5–1.0 µg
Bacterial genomic DNA	2–3 µg

Sequencing templates should be purified before use in sequencing reactions. See <https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/dna-extraction.html> for a range of suitable kits.

Perform cycle sequencing

Set up the sequencing reactions

IMPORTANT! Protect dye terminators from light. Cover the reaction mix and sequencing plates with aluminum foil before use.

1. Completely thaw the contents of the BigDye™ Terminator v3.1 Cycle Sequencing Kit and your primers and store on ice.
2. Vortex the tubes for 2 to 3 seconds, then centrifuge briefly (2 to 3 seconds) with a benchtop microcentrifuge to collect contents at the bottom of the tubes.
3. Label microcentrifuge tubes “forward” and “reverse” and add components as indicated:

IMPORTANT! Change pipette tips after each transfer.

IMPORTANT! For control reactions use 4 µL of the control primers (0.8pmol/µL) in both 10 µL and 20 µL reactions.

Component	Standard reaction (20 µL) ^[1]		
	Quantity per reaction	Example Forward	Example Reverse
BigDye™ Terminator 3.1 Ready Reaction Mix	8 µL	8 µL	8 µL
Forward primer (3.2 µM)	3.2 pmol	1 µL	—
Reverse primer (3.2 µM)		—	1 µL
Deionized water (RNase/DNase-free)	Varies based on template and primer volume	9 µL	9 µL
Template	See “Template quantity” on page 1	2 µL ^{[2], [3]}	2 µL ^{[2], [3]}
Total volume	20 µL	20 µL	20 µL

^[1] Reactions can be scaled to 10 µL for 384-well plates. Keep the primer concentration and volume the same as in 20 µL reactions.

^[2] e.g., 150–300ng/µL of dsDNA

^[3] Concentration of template may affect volume, if template volume differs please adjust the volume of water in the reaction mix.

Note: Store on ice and protected from light.

4. Seal the plate with MicroAmp™ Clear Adhesive Film.
5. Vortex the plate for 2 to 3 seconds, then centrifuge briefly in a swinging bucket centrifuge to collect contents to the bottom of the wells (5 to 10 seconds) at 1,000 x g.

Note: Bubbles may be present within the wells, but do not adversely affect the reaction.

Using BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer to dilute sequencing reactions

Some cycle sequence reactions may be optimized using diluted BigDye™ Terminator Ready Reaction Mix. The BigDye™ Terminator Ready Reaction Mix is provided at a 2.5X concentration and can be diluted using BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer to a final end reaction concentration of 1X.

Calculate the volume of BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer to use:

$0.5 * ((\text{total reaction volume})/2.5) - \text{volume of BigDye™ Terminator Ready Reaction Mix}$.

Note: If you use the BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer without optimization, the quality of the sequence may deteriorate. We can not guarantee the performance of BigDye™ chemistry when it is diluted.

An example of a 0.5x diluted sequencing reaction is shown below:

Component	Diluted reaction (0.5X)		
	Quantity per reaction	Example Forward	Example Reverse
BigDye™ Terminator 3.1 Ready Reaction Mix	4 µL	4 µL	4 µL
BigDye™ Terminator v1.1 & v3.1 5X Sequencing Buffer	2 µL	2 µL	2 µL
Forward primer (3.2 µM)	3.2 pmol	1 µL	—
Reverse primer (3.2 µM)		—	1 µL
Deionized water (RNase/DNase-free)	Varies based on template and primer volume	11 µL	11 µL
Template	See "Template quantity" on page 1	2 µL ^{[1],[2]}	2 µL ^{[1],[2]}
Total volume	20 µL	20 µL	20 µL

^[1] e.g., 150-300ng/µL of dsDNA

^[2] Concentration of template may affect volume, if template volume differs please adjust the volume of water in the reaction mix.

Run the sequencing reactions

- Place the tubes or plate(s) in a thermal cycler and set the volume.
- Perform cycle sequencing:

Parameter	Stage/step				
	Incubate	Cycling (25 cycles)			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second.			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	01:00	00:10	00:05	04:00 ^[1]	Until ready to purify.

^[1] Shorter extension times can be used for short templates.

Purify the sequencing reactions

Salts, unincorporated dye terminators, and dNTPs in sequencing reactions obscure data in the early part of the sequence and can interfere with basecalling.

Purify the sequencing reactions before capillary electrophoresis. See the *BigDye™ Terminator v3.1 Cycle Sequencing Kit User Guide* (Pub. No. 4337035) for recommended protocols.

Capillary electrophoresis

Capillary electrophoresis guidelines

- Resuspend sequencing reactions in 10-µL of Hi-Di™ Formamide. Do not heat samples to resuspend. Run samples as soon as possible after resuspension.

Note: It is not necessary to resuspend samples purified with the BigDye XTerminator™ Purification Kit.

- Select the correct mobility file. Different dyes will have different mobility corrections required for adequate basecalling.

If the wrong mobility file is used, this can be corrected with Sequencing Analysis Software.

Compatible sequencing instruments

- 310 Genetic Analyzer
- 3130/3130xl Genetic Analyzer
- 3500/3500xL Genetic Analyzer
- 3730/3730xl DNA Analyzer

Calibration

Matrix or sequencing standards provide a sample for multi-color spectral correction for the dye emission overlap of the BigDye™ Terminators.

Perform new spectral calibrations when an array is installed or capillaries are moved within the detection area to ensure and maintain the highest quality spectral calibration on your system.

See your specific instrument user guide for more information on calibration.

The information in this guide is subject to change without notice.

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