# BigDye™ Terminator v1.1 Cycle Sequencing Kit

**Catalog Numbers** 4337449, 4337450, 4337451, 4337452

**Pub. No.** MAN0015667 **Rev.** A.0

**Note:** For safety and biohazard guidelines, see the "Safety" appendix in the *BigDye™ Terminator v1.1 Cycle Sequencing Kit User Guide* (Pub. no. 4337036). 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 v1.1 Cycle Sequencing Kit (Cat. Nos. 4337449, 4337450, 4337451, and 4337452). See the *BigDye™ Terminator v1.1 Cycle Sequencing Kit User Guide* (Pub. No. 4337036) for detailed instructions and troubleshooting.

## Product description

The BigDye™ Terminator v1.1 Cycle Sequencing Kit provides the reagents required for Sanger sequencing reactions in a pre-mixed format.

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

The kit has been designed to deliver optimal 5' resolution and basecalling in shorter fragments when used in combination with POP-6™ polymer and a 50cm array. 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 v1.1 Sequencing Standard 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 v1.1 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 * ((total reaction volume)/2.5) - 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 v1.1 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

1. Place the tubes or plate(s) in a thermal cycler and set the volume.
2. 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 v1.1 Cycle Sequencing Kit User Guide* (Pub. No. 4337036) 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
- 3130/3130*xl* Genetic Analyzer
- 3500/3500xL Genetic Analyzer
- 3730/3730*xl* 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.



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