BigDye XTerminator™ Purification Kit

Catalog Numbers 4376484, 4376485, 4376486, and 4376487

Pub. No. 4383427 Rev. C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of the BigDye XTerminator™ Purification Kit. For detailed instructions, supplemental procedures, and troubleshooting, see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).

Important guidelines

- **IMPORTANT!** Keep the reagents thoroughly mixed throughout the pipetting procedure.
- For optimal results, follow "Guidelines for sequencing reactions" on page 3.
- Use wide-bore tips (with an orifice >1.0 mm) to pipet the BigDye XTerminator™ Solution.
- Use conventional tips to pipet the SAM™ Solution and Premix Solution.
- Ensure the pipette tip is below the surface of the liquid when pipetting the BigDye XTerminator™ Solution.
- Perform all vortexing steps as described in the procedure.
- Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents.

Before you begin

- Inspect the SAM™ Solution. If precipitates are visible, warm the solution at 37°C, then gently mix to dissolve the precipitates. Cool the solution to room temperature before use.
- Thoroughly mix the SAM™ Solution.
- If you are using the direct injection method, ensure the appropriate BDx run module is installed and updated on your system (see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408)).

Sequential Pipetting method: Add the reagents to the reaction plate

Sequentially add the reagents to the sequencing reactions

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.
2. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.
3. Add the following components (in the order indicated) to each well of the reaction plate.

   IMPORTANT! Remix the BigDye XTerminator™ Solution every minute to prevent phase separation.

Component	Volume per 5-µL reaction	Volume per 10-µL reaction	Volume per 20-µL reaction
SAM™ Solution	22.5 µL	45 µL	90 µL
BigDye XTerminator™ Solution	5 µL	10 µL	20 µL

4. Seal the plate according to your sealing method.
   - Using a MicroAmp™ Clear Adhesive Film—Proceed to "Seal the reaction plate" on page 2.
   - Using a heat seal—Apply a heat seal at 160°C for 1.5 seconds, then proceed to "Vortex, then centrifuge the reaction plate" on page 3.

Premix Pipetting method: Add the reagents to the reaction plate

Prepare the Premix Solution

Prepare fresh Premix Solution for each processing run.

1. Immediately before pipetting, mix the BigDye XTerminator™ Solution by inversion 10 times or until the solution is homogeneous.

2. Combine the following components (in the order indicated) in an appropriately-sized bottle or reagent reservoir according to one of the following tables.

Table 1 384-well plate, 5-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	5 µL	2,304 µL
SAM™ Solution	22.5 µL	10,368 µL
Total Premix Solution	**27.5 µL**	**12,672 µL**

[1] Includes 20% overage.

Table 2 96-well plate, 10-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	10 µL	1,152 µL
SAM™ Solution	45 µL	5,184 µL
Total Premix Solution	**55 µL**	**6,336 µL**

[1] Includes 20% overage.

Table 3 96-well plate, 20-µL reaction volume

Component	Volume	
	1 well	1 plate[1]
BigDye XTerminator™ Solution	20 µL	2,304 µL
SAM™ Solution	90 µL	10,368 µL
Total Premix Solution	**110 µL**	**12,672 µL**

[1] Includes 20% overage.

Add the Premix Solution to the sequencing reactions

IMPORTANT! Keep the Premix Solution thoroughly mixed throughout the pipetting procedure.

1. Centrifuge the cycle-sequencing reaction plate at 1,000 × *g* for 1 minute.

2. Immediately before pipetting, mix the Premix Solution.
   - For Premix Solution in a bottle—Cap the bottle, then mix by inversion 10 times or until the solution is homogeneous.
   - For Premix Solution in a reagent reservoir—Pipet up and down 2–3 times or until the solution is homogeneous.

3. Add the Premix Solution to each well of the reaction plate according to the following table.

IMPORTANT! Agitate the solution before each aspiration.

Plate type (reaction volume per well)	Volume of Premix Solution per well
384-well (5-µL)	27.5 µL
96-well (10-µL)	55 µL
96-well (20-µL)	110 µL

Discard any remaining Premix Solution after use.

4. Seal the plate according to your sealing method.
   - Using a MicroAmp™ Clear Adhesive Film—Proceed to "Seal the reaction plate" on page 2.
   - Using a heat seal—Apply a heat seal at 160℃ for 1.5 seconds, then proceed to "Vortex, then centrifuge the reaction plate" on page 3.

Seal the reaction plate

IMPORTANT! Apply firm pressure to the adhesive film during application to ensure a tight, leak-proof seal during vortexing.

1. Place the reaction plate on a MicroAmp™ Splash-Free 96-Well Base, then wipe off any liquid on the surface of the plate.

2. Place the adhesive film onto the reaction plate (adhesive side facing the plate).

3. While applying firm downward pressure, move the applicator slowly across the film, both horizontally and vertically.

4. Repeat step 3 five times. While applying pressure, run the edge of the applicator along all four sides of the outer border of the film.

5. Inspect the reaction plate to confirm that all wells are sealed.

The plate is properly sealed when an imprint of each well is visible on the surface of the film.

Vortex, then centrifuge the reaction plate

1. Firmly attach the plate to the vortexer.

   For set-up and operating instructions specific to your instrument, see the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).

2. Vortex the plate for 20 minutes (96-well plate) or 30 minutes (384-well plate), using the settings indicated.

   IMPORTANT! Do not vortex beyond the specified time.

Vortexer	Plate type	Speed or setting
Thermo Scientific™ Digital Vortex Mixer	96-well	1,800 rpm
	384-well	2,000 rpm
Digital Vortex-Genie™ 2	96-well	1,800 rpm
	384-well	2,000 rpm
Eppendorf™ MixMate™	96-well	1,800 rpm
	384-well	2,600 rpm
IKA™ MS 3 Digital Orbital Shaker	96-well or 384-well	1,800 rpm (Mode B)
IKA™ Vortex 3	96-well or 384-well	Setting 5[1]
Taitec MicroMixer E-36	96-well or 384-well	Maximum
Union Scientific™ Vertical Shaker[2]	96-well or 384-well	Setting 100

[1] Use the maximum setting that does not cause the vortexer to become unstable.
[2] If needed, add additional plates to meet the mass requirements.

3. Centrifuge the plate at 1,000 × *g* in a swinging-bucket centrifuge for 2 minutes.

4. Immediately proceed to capillary electrophoresis.

STOPPING POINT If you cannot run capillary electrophoresis immediately, store the reaction plate as described (see "Guidelines for storing the reaction plate" on page 3).

Prepare the reaction plate for capillary electrophoresis

Prepare the reaction plate according to the following table.

IMPORTANT! Do not heat-denature or use Hi-Di™ Formamide with samples that contain BigDye XTerminator™ reagents.

Instrument	Action
SeqStudio™	**For 96-well plates—**Remove the adhesive film, then place a septum on the plate.
3500/3500xL	**For 96- or 384-well plates—**Remove the adhesive film, then place a septum on the plate.
3730/3730*xl*	**For plates sealed with heat seal film—**Proceed with the sealed plate.
	For plates sealed with adhesive film—Prepare the plate according to the plate type. • **For 96-well plates—**Remove the adhesive film, then place a septum on the plate. • **For 384-well plates—**Remove the adhesive film, then perform one of the following actions. – Apply a heat seal to the plate. – Transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.
3130/3130*xl*	• **For 96-well plates—**Remove the adhesive film, then place a septum on the plate. • **For 384-well plates—**Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.
310 Genetic Analyzer	**For 96-well trays—**Remove the adhesive film, transfer 10 µL of the supernatant to a new plate, then place a septum on the plate.

Run capillary electrophoresis

1. Load the reaction plate in the instrument.

2. Set up an instrument run using the appropriate conditions for your instrument.
   - If you are using the direct injection method, use a BDx run module specified in the *BigDye XTerminator™ Purification Kit User Guide* (Pub. No. 4374408).
   - If the supernatant was transferred to a new plate after purification, see your instrument user guide for the appropriate run module.

3. Start the electrophoresis run.

(Optional) Store reaction plates that have been at room temperature <48 hours as described (see "Guidelines for storing the reaction plate" on page 3).

Guidelines for storing the reaction plate

Sequencing reactions purified with the BigDye XTerminator™ Purification Kit are stable at room temperature for up to 48 hours.

Store reaction plates covered with adhesive film, septa, or heat seal film at room temperature for up to 48 hours.

Note: After removing the reaction plate from storage, centrifuge the plate at 1,000 × *g* in a swinging-bucket rotor for 2 minutes, then proceed to run capillary electrophoresis.

Guidelines for sequencing reactions

- DNA sequencing reactions that are purified with the BigDye XTerminator™ Purification Kit result in high signal strength when analyzed. If needed, decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on-scale during analysis.

  Note: If the template concentration is decreased, the amount of any template controls must be decreased proportionately.

- Ensure the sequencing reactions meet the minimum volume requirements according to the following table.

If needed, adjust the reaction volume with UltraPure™ DNase/RNase-Free Distilled Water before purification with the kit.

Plate type	Minimum reaction volume
384-well	5 µL
96-well	10 µL[1]

[1] If you are processing >48 samples for a single capillary electrophoresis run, we recommend a minimum reaction volume of 20 µL.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

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Revision	Date	Description
C	2 June 2020	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Updated compatible instruments. • Added detailed procedure to seal the reaction plate. • Made wording and formatting updates.
B	July 2007	Baseline for this revision history.