

BigDye® XTerminator™ Purification Kit

- Single-well plate—No liquid transfer
- Complete dye blob removal
- Stabilized samples
- Improves any sequencing workflow
- Manual or automated protocols
- Rapid, reliable, and reproducible

The BigDye[®] XTerminator[™] Purification Kit is a fast, simple purification method for DNA sequencing reactions that improves the sequencing workflow (Figure 1) and removes unincorporated BigDye terminators. No more dye blobs! Cleanup is complete in under 40 minutes and requires less than 10 minutes of labor.

More effective than ethanol precipitation (no disappearing pellets!), and cheaper than column purification and other commercial kits, the BigDye XTerminator Purification Kit removes dye blobs by capturing unincorporated dye terminators, salts and other charged molecules that may interfere with base calling and electrokinetic sample injection (Figures 2–4). The kit is compatible with all terminator sequencing chemistries, reaction volumes, and template types.

Simple Purification Process

Traditional purification methods, such as ethanol precipitation, require the addition of multiple reagents along with decanting and centrifuging steps.

The BigDye XTerminator Purification Kit requires the addition of only two reagents, which can be added sequentially or premixed:

- XTerminator[™] Solution—Scavenges unincorporated dye terminators and free salts from the post-sequencing reaction
- SAM[™] Solution—Enhances the performance of the XTerminator[™] Solution and stabilizes the postpurification reactions



Figure 2. High Quality, Long Read Sequence Data Using the BigDye XTerminator Purification Kit. High quality dye blob-free data can be produced using BigDye XTerminator Purification Kit without sacrifice to sequence length of read. Q20 base read = 1044 bp. Data kindly provided by the DNA Technology Unit, Plant Biotechnology Institute (NRC-PBI), Saskatoon, Canada.

Simplified Sequencing Workflow

Perform cycle sequencing with BigDye® terminator chemistry



Figure 1. Simplified and faster sequencing workflow using the BigDye[®] XTerminator[™] Purification Kit.



Figure 3. Purification using BigDye XTerminator Kit versus Ethanol Precipitation (A) BigDye XTerminator Purification Kit yields dye blob-free data. (B) Ethanol purification generally yields sequence data with dye blobs.

Simplified Sequencing Workflow

STEP 1. Perform cycle sequencing with BigDye[®] terminators.

STEP 2. After cycle sequencing, centrifuge the reaction plate briefly, then pipette the SAM[™] Solution into each well, per the table below.

Note: XTerminator[™] and SAM[™] solutions may be premixed and added in a single step. For details, please see Big Dye[®] XTerminator[™] Purification Kit Protocol (P/N 4374408).

Sequencing Reaction Volume	SAM Solution (µL/well)	
10 µL/well (96-well plate)	45	
20 µL/well (96-well plate)	90	

STEP 3. Pipette the correct volume of XTerminator Solution into each well.

- a. Vortex the XTerminator Solution bulk container briefly.
- b. Using a wide-bore pipette tip, aspirate the solution per the table below and add to each well.

	BigDye	
Sequencing	XTerminator	
Reaction Volume	Solution (µL/well)	
10 µL/well (96-well plate)	10	
20 µL/well (96-well plate)	20	



Figure 4. (A) BigDye XTerminator Purification yields high quality data and superior short fragment recovery and removes dye blobs from unincorporated dye terminators. (B) Traditional methods yield sequence data with dye blobs. Data kindly provided by the DNA Technology Unit, Plant Biotechnology Institute (NRC-PBI), Saskatoon, Canada.

 c. Repeat steps "a" through "b" to pipette the complete volume of XTerminator Solution into each well.

STEP 4. Seal the plate using a heat seal or Clear Adhesive Film (P/N 4306311). Vortex for 30 minutes, then centrifuge the reaction plate briefly.

STEP 5. Place the reaction plate in the Applied Biosystems DNA analyzer. Select run module and run plate.

Note: This protocol can be automated on a Biomek® FX Laboratory Automation Workstation (Beckman Coulter). For details please see Big Dye XTerminator Purification Kit Protocol (P/N 4374408).

Software

Applied Biosystems provides downloadable run modules for use with the Big Dye XTerminator Purification Kit and Data Collection Software. These modules are designed for the 3100, 3100–*Avant*, 3130/3130*xl*, and 3730/3730*xl* Analyzers.

The software modules for the BigDye XTerminator Purification Kit are compatible with the Windows® NT, Windows® 2000, and Windows® XP operating systems and are available for download at www2.appliedbiosystems. com/support/software/.

Product Description	Package	Part Number
BigDye XTerminator Purification Kit	2 mL (~100 x 20 µL purifications)	4376486
	20 mL (~1,000 x 20 µL purifications)	4376487
	50 mL (~2,500 x 20 µL purifications)	4376484

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