

Quick Reference Card

For safety and biohazard guidelines, refer to the “Safety” section in the *Applied Biosystems BigDye® XTerminator™ Purification Kit Protocol* (PN 4374408). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Overview

The BigDye® XTerminator™ Purification Kit sequesters cycle-sequencing reaction components such as salt ions, unincorporated dye terminators, and dNTPs to prevent their coinjection with dye-labeled extension products into a CE DNA analyzer. The BigDye® XTerminator™ reagents can be pipetted separately and sequentially into a reaction plate, or premixed together before being pipetted into a reaction plate.

Ordering Information

Refer to the *BigDye® XTerminator™ Purification Kit Protocol* for recommended vortexers and required accessories.

Kit Size	Approximate Number of 20- μ L Reactions	Volume of Each Kit Reagent (mL)		Part Number
		XTerminator™ Solution	SAM™ Solution	
2-mL	100	2	9	4376486
20-mL	1,000	20	90	4376487
50-mL	2,500	50	225	4376484
800-mL	40,000	800	3600	4376485

Important Tips

- When you pipette directly from the XTerminator Solution bottle:
 - Before pipetting, mix the XTerminator Solution until homogeneous.
 - Use wide-bore pipette tips.
 - Avoid pipetting near the surface of the liquid.
 - When you seal the reaction plate, verify that each well is sealed.
- To achieve optimum performance, use a recommended vortexer and follow the protocol when you vortex the reaction plate.
- When you load plates into the CE instrument:
 - Do not heat-denature or use Hi-Di™ Formamide with samples containing BigDye XTerminator reagents.
 - Use the BigDye XTerminator Purification Kit run modules specified for your instrument and plate type. The run modules are available at www.appliedbiosystems.com. Click **Support**, then **Software Downloads**. In the list, select **BigDye® XTerminator™ Purification Kit**.

Procedure for Sequential Pipetting

STEP	ACTION									
1	Centrifuge the sequencing reaction plates.	<p>Follow the cycle-sequencing protocol. When the reaction is complete, centrifuge the reaction plate for 1 minute to spin down plate contents.</p> <p>IMPORTANT! You may need to decrease the amount of DNA template in the sequencing reactions to compensate for increased signal strength. See “DNA Quantity Guidelines” on page 6.</p>								
2	Add the SAM™ Solution to the reaction plates.	<p>To each well of the reaction plate, add the volume of SAM Solution specified below, <i>using a conventional pipette tip</i>.</p> <p>Make sure there are no particulates in the SAM Solution before pipetting. If particulates are present, heat the SAM Solution to 37 °C and mix to redissolve. Cool to room temperature before using.</p> <table border="1" data-bbox="387 531 1241 718"> <thead> <tr> <th>Plate Type and Reaction Volume/Well</th> <th>Volume of SAM™ Solution/Well (µL)</th> </tr> </thead> <tbody> <tr> <td>384-well, 5-µL</td> <td>22.5</td> </tr> <tr> <td>96-well, 10-µL</td> <td>45.0</td> </tr> <tr> <td>96-well, 20-µL</td> <td>90.0</td> </tr> </tbody> </table> <p>IMPORTANT! For 384-well reactions with reaction volumes less than 5 µL, add water to bring the volumes to 5 µL before adding the SAM Solution. For 96-well reactions with reaction volume less than 10 µL, add water to bring the volume to 10 µL before adding the SAM Solution.</p>	Plate Type and Reaction Volume/Well	Volume of SAM™ Solution /Well (µL)	384-well, 5-µL	22.5	96-well, 10-µL	45.0	96-well, 20-µL	90.0
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3	Add the XTerminator™ Solution to the reaction plates, using a wide-bore pipette tip.	<p>Add the XTerminator Solution:</p> <ol style="list-style-type: none"> Vortex the XTerminator Solution bulk container at maximum speed for at least 10 seconds, until it is homogeneous. <i>Using a wide-bore pipette tip</i>, add to the reaction plate the volume of XTerminator Solution specified below. <table border="1" data-bbox="448 1008 1260 1220"> <thead> <tr> <th>Plate Type and Reaction Volume/Well</th> <th>Volume of XTerminator™ Solution/Well (µL)</th> </tr> </thead> <tbody> <tr> <td>384-well, 5-µL</td> <td>5.0</td> </tr> <tr> <td>96-well, 10-µL</td> <td>10.0</td> </tr> <tr> <td>96-well, 20-µL</td> <td>20.0</td> </tr> </tbody> </table>	Plate Type and Reaction Volume/Well	Volume of XTerminator™ Solution/Well (µL)	384-well, 5-µL	5.0	96-well, 10-µL	10.0	96-well, 20-µL	20.0
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4	Seal, vortex, load, and run the plates.	Follow the instructions in “After Pipetting Is Complete” on page 4.								

Procedure for Premix Pipetting

Note: The premix is stable only for 5 days. Make only the volume of premix that you will use in 5 days.

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<p>1</p>	<p>Calculate the required volume of the XTerminator reagents.</p>	<p>Based on your plate and reaction size, calculate the volume of SAM Solution and XTerminator Solution needed.</p> <p>Note: All volumes below include an additional 10% to account for dead volume in the reagent trough.</p> <p>For 384-well plate, 5-μL reactions:</p> <table border="1" data-bbox="395 435 1266 600"> <thead> <tr> <th>Reagent</th> <th>Volume/Well (μL)</th> <th>Volume/Plate (μL)</th> <th>Number of Reactions</th> <th>Final Volume Needed</th> </tr> </thead> <tbody> <tr> <td>SAM Solution</td> <td>24.75</td> <td>9504</td> <td></td> <td></td> </tr> <tr> <td>XTerminator Solution</td> <td>5.5</td> <td>2112</td> <td></td> <td></td> </tr> </tbody> </table> <p>For 96-well plate, 10-μL reactions:</p> <table border="1" data-bbox="395 664 1266 829"> <thead> <tr> <th>Reagent</th> <th>Volume/Well (μL)</th> <th>Volume/Plate (μL)</th> <th>Number of Reactions</th> <th>Final Volume Needed</th> </tr> </thead> <tbody> <tr> <td>SAM Solution</td> <td>49.5</td> <td>4752</td> <td></td> <td></td> </tr> <tr> <td>XTerminator Solution</td> <td>11</td> <td>1056</td> <td></td> <td></td> </tr> </tbody> </table> <p>For 96-well plate, 20-μL reactions:</p> <table border="1" data-bbox="395 894 1266 1058"> <thead> <tr> <th>Reagent</th> <th>Volume/Well (μL)</th> <th>Volume/Plate (μL)</th> <th>Number of Reactions</th> <th>Final Volume Needed</th> </tr> </thead> <tbody> <tr> <td>SAM Solution</td> <td>99</td> <td>9504</td> <td></td> <td></td> </tr> <tr> <td>XTerminator Solution</td> <td>22</td> <td>2112</td> <td></td> <td></td> </tr> </tbody> </table>				Reagent	Volume/Well (μ L)	Volume/Plate (μ L)	Number of Reactions	Final Volume Needed	SAM Solution	24.75	9504			XTerminator Solution	5.5	2112			Reagent	Volume/Well (μ L)	Volume/Plate (μ L)	Number of Reactions	Final Volume Needed	SAM Solution	49.5	4752			XTerminator Solution	11	1056			Reagent	Volume/Well (μ L)	Volume/Plate (μ L)	Number of Reactions	Final Volume Needed	SAM Solution	99	9504			XTerminator Solution	22	2112		
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<p>2</p>	<p>Combine the reagents to create the premix.</p>	<p>Combine the SAM Solution and the XTerminator Solution:</p> <ol style="list-style-type: none"> Vortex the XTerminator Solution bulk container at maximum speed for at least 10 seconds, until it is homogeneous. Using a wide-bore pipette tip or a graduated cylinder, add the appropriate volume of XTerminator Solution to a clean container. IMPORTANT! Avoid pipetting near the surface of the liquid. Using a conventional pipette tip or a graduated cylinder, add the appropriate volume of the SAM Solution to the container with the XTerminator Solution. Make sure there are no particulates in the SAM Solution before pipetting. If particulates are present, heat the SAM Solution to 37 °C and mix to redissolve. Cool to room temperature before using. Mix the reagents until homogeneous. <p>Note: The premix can be stored in a clean, capped container at 4 °C for up to 5 days.</p>																																																

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3	Centrifuge the sequencing reaction plates.	Follow the cycle-sequencing protocol. When the reaction is complete, centrifuge the reaction plate for 1 minute to spin down plate contents. IMPORTANT! You may need to decrease the amount of DNA template in the sequencing reactions to compensate for increased signal strength. See “DNA Quantity Guidelines” on page 6.								
4	Add the premix to the reaction plates.	Using a conventional pipette tip, add to each well of the reaction plate the volume of the thoroughly mixed premix specified below. IMPORTANT! For 384-well reactions with reaction volumes less than 5 μL , add water to bring the volumes to 5 μL before adding the premix. For 96-well reactions with reaction volume less than 10 μL , add water to bring the volume to 10 μL before adding the premix. <table border="1" data-bbox="396 487 1264 673"> <thead> <tr> <th>Plate Type and Reaction Volume/Well</th> <th>Volume of Premix/Well (μL)</th> </tr> </thead> <tbody> <tr> <td>384-well, 5-μL</td> <td>27.5</td> </tr> <tr> <td>96-well, 10-μL</td> <td>55.0</td> </tr> <tr> <td>96-well, 20-μL</td> <td>110.0</td> </tr> </tbody> </table> IMPORTANT! Mix the premix as needed to maintain a homogeneous solution. Dispense the premix within 1 minute of aspiration to avoid separation of the reagents in the pipette tip.	Plate Type and Reaction Volume/Well	Volume of Premix/Well (μL)	384-well, 5- μL	27.5	96-well, 10- μL	55.0	96-well, 20- μL	110.0
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After Pipetting Is Complete

STEP	ACTION	
1	Seal the reaction plates.	Seal the plate, using: <ul style="list-style-type: none"> A heat seal at 160 °C for 2 seconds. or MicroAmp™ Clear Adhesive Films. (See “Appendix C: Plate Sealing Procedure,” in the <i>BigDye® X Terminator™ Purification Kit Protocol</i> for details.) Verify that each well is sealed. IMPORTANT! If you are using an Applied Biosystems 3730/3730x/ DNA Analyzer and plan to use direct injection, only Applied Biosystems Heat Seal Film for Sequencing and Fragment Analysis Sample Plates (PN 4337570) is supported.

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<p>2</p>	<p>Vortex the reaction plates.</p>	<p>Vortex the reaction plate for 30 minutes using the following conditions:</p> <table border="1" data-bbox="395 222 1201 569"> <thead> <tr> <th>Vortexer</th> <th>Plate Type</th> <th>Speed</th> </tr> </thead> <tbody> <tr> <td rowspan="2">Digital Vortex-Genie 2</td> <td>96-well</td> <td>1800 rpm</td> </tr> <tr> <td>384-well</td> <td>2000 rpm</td> </tr> <tr> <td>Eppendorf MixMate</td> <td>384-well</td> <td>2600 rpm</td> </tr> <tr> <td>IKA MS3 Digital</td> <td>Either</td> <td>2000 rpm[‡]</td> </tr> <tr> <td>IKA Vortex 3</td> <td>Either</td> <td>Setting 5[§]</td> </tr> <tr> <td>Taitec MicroMixer E-36</td> <td>Either</td> <td>Maximum</td> </tr> <tr> <td>Union Scientific Vertical Shaker[#]</td> <td>Either</td> <td>Setting 100</td> </tr> </tbody> </table> <p> [‡] Set the vortexer to Mode B. (See the <i>BigDye[®] XTerminator[™] Purification Kit Protocol</i> for instructions.) [§] Use the maximum setting that does not cause the vortexer to “walk” across the bench. [#] Add plates needed to meet mass requirements. (See the <i>BigDye[®] XTerminator[™] Purification Kit Protocol</i> for information.) </p> <p>Note: It is recommended that you pause vortexing after 1 minute to verify that the contents are well mixed.</p>		Vortexer	Plate Type	Speed	Digital Vortex-Genie 2	96-well	1800 rpm	384-well	2000 rpm	Eppendorf MixMate	384-well	2600 rpm	IKA MS3 Digital	Either	2000 rpm [‡]	IKA Vortex 3	Either	Setting 5 [§]	Taitec MicroMixer E-36	Either	Maximum	Union Scientific Vertical Shaker [#]	Either	Setting 100			
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<p>3</p>	<p>Centrifuge the reaction plates.</p>	<p>In a swinging-bucket centrifuge, spin the plate at 1000 × g for 2 minutes.</p>																											
<p>4</p>	<p>Prepare the plates for the instrument run.</p>	<p>Place the reaction plate in the CE instrument. (To store and run the plate later, see step 6.)</p> <table border="1" data-bbox="395 869 1259 1541"> <thead> <tr> <th>Plate Type</th> <th>Instrument</th> <th>Seal</th> <th>Instructions</th> </tr> </thead> <tbody> <tr> <td rowspan="2">384-well</td> <td rowspan="2">3730/3730<i>xl</i></td> <td>Heat seal</td> <td>Place directly in the instrument.</td> </tr> <tr> <td>MicroAmp[™] Clear Adhesive Film</td> <td> <ul style="list-style-type: none"> Remove the clear adhesive film, replace with a heat seal, then place in the instrument. or Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, place in instrument. </td> </tr> <tr> <td rowspan="4">96-well</td> <td rowspan="2">3730/3730<i>xl</i></td> <td>Heat seal</td> <td>Place directly in the instrument.</td> </tr> <tr> <td>MicroAmp Clear Adhesive Film</td> <td>Remove the seal, replace with a septa mat, place in the instrument.</td> </tr> <tr> <td>3100/3100 <i>Avant</i>[‡], 3130/3130<i>xl</i>, or 310 Genetic Analyzer</td> <td>Either</td> <td>Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, then place in the instrument.</td> </tr> <tr> <td>3100/3100 <i>Avant</i>[‡] or 3130/3130<i>xl</i></td> <td>Either</td> <td>Remove seal, replace with a septa mat, then place in the instrument.</td> </tr> <tr> <td></td> <td>310 Genetic Analyzer</td> <td>Either</td> <td>Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, then place in the instrument.</td> </tr> </tbody> </table> <p> [‡] The instrument must be using Data Collection Software v2.0 or later. If not, transfer 10 µL of supernatant to a clean plate, cover with a septa mat, place in the instrument. </p>		Plate Type	Instrument	Seal	Instructions	384-well	3730/3730 <i>xl</i>	Heat seal	Place directly in the instrument.	MicroAmp [™] Clear Adhesive Film	<ul style="list-style-type: none"> Remove the clear adhesive film, replace with a heat seal, then place in the instrument. or Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, place in instrument. 	96-well	3730/3730 <i>xl</i>	Heat seal	Place directly in the instrument.	MicroAmp Clear Adhesive Film	Remove the seal, replace with a septa mat, place in the instrument.	3100/3100 <i>Avant</i> [‡] , 3130/3130 <i>xl</i> , or 310 Genetic Analyzer	Either	Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, then place in the instrument.	3100/3100 <i>Avant</i> [‡] or 3130/3130 <i>xl</i>	Either	Remove seal, replace with a septa mat, then place in the instrument.		310 Genetic Analyzer	Either	Transfer 10 µL of supernatant to a clean plate, cover with a septa mat, then place in the instrument.
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STEP	ACTION	
5	Select the appropriate run module.	Select the appropriate BigDye XTerminator run module for your instrument and plate type. Note: Use standard run modules if you transferred the supernatant to a clean plate after centrifuging.
6	Run the reaction plates.	Run the plate. If the reaction plates are not run immediately, you can store them under the following conditions: <ul style="list-style-type: none"> • Room temperature – Plates sealed with heat seal film, adhesive film, or septa for up to 48 hours at room temperature (20 to 25 °C). • Refrigerated storage – Plates sealed with heat seal film or adhesive film for up to 10 days at 4 °C (recommended). • Frozen storage – Plates sealed with heat seal film or adhesive film for up to 10 days at –20 °C.

DNA Quantity Guidelines

DNA sequencing reactions purified with the BigDye® XTerminator™ Purification Kit result in high signal strength when analyzed on a DNA sequencer. Therefore, when you prepare sequencing samples for purification with the BigDye XTerminator reagents, you may need to decrease the amount of DNA template in the sequencing reactions to keep the fluorescence signals on scale during analysis. Use the following table as a guide to the amount of template DNA for the initial cycle sequencing.

IMPORTANT! If you decrease the template concentration, also decrease the amount of any template controls proportionately. For example, if you run a pGEM control, dilute it 1:2 or 1:4 and add only 1 to 2 µL.

Template Type	DNA Quantity/Reaction (ng)	Template Type	DNA Quantity/Reaction (ng)
PCR products		Other types of template	
100 to 200 bp	0.5 to 3	Single-stranded DNA	10 to 50
200 to 500 bp	1 to 10	Double-stranded DNA	50 to 300
500 to 1000 bp	2 to 20	Cosmid or BAC DNA	200 to 1,000
1000 to 2000 bp	5 to 40	Bacterial genomic DNA	1,000 to 3,000
>2000 bp	10 to 50		

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