XT Purification Kit User Manual

Sequencing workflow

- ✧ STEP 1: Add XT Purification Reagents
- ✧ STEP 2: Seal reaction plate
- ✧ STEP 3: Vortex for 30 minutes
- ✧ STEP 4: Centrifuge

Run purified samples on your DNA Analyzer

Overview

The XT Purification Kit sequesters cycle-sequencing reaction components such as salt Ions,unincorporated dye terminators, and dNTPs to prevent their co-injection with dye-labeled extension products into a CE DNA analyzer. The XT Purification reagents can be pipette separately and sequentially into reaction plate, or premixed together before being pipette into a reaction plate.

Ordering Information

Refer to the XT Purification Kit Protocol for recommended vortexers and required accessories.

Kit Size	Volume of Each Kit Reagent (ml)		Catalog Number
	Resin	Solution	
50preps	1ml	4.5ml	8500050
1000preps	20ml	90ml	8502010
5000preps	100ml	450ml	8502050

Important Tips

- ✧ When you pipette directly from the Solution bottle:
  Before pipetting, mix the 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 Formamide with samples containing XT Purification reagents.
  Use the ABI run modules specified for your instrument and plate type.

Procedure for Sequential Pipetting

STEP	ACTION	
1	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 5.

<table>
<tr><td></td><td></td><td></td></tr>
<tr><td>2</td><td>Add the Solution to the reaction plates</td><td>To each well of the reaction plate, add the volume of the Solution specified below, using a conventional pipette tip.
Make sure there are no particulates in the Solution before pipetting. If particulates are present, heat the Solution to 37 ℃ and mix to redissolve. Cool to room temperature before using.
<table>
<tr><th>Plate Type and Reaction Volume/Well</th><th>Volume of the Solution/Well (µl)</th></tr>
<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>
</table>
IMPORTANT! For 384-well reactions with reaction volumes less than 5 µl, add water to bring the volumes to 5 µl before adding the Solution. For 96-well reactions with reaction volumes less than 10 µl, add water to bring the volume to 10 µl before adding the solution.</td></tr>
<tr><td>3</td><td>Add the Resin to the reaction plates using a wide-bore pipette tips</td><td>Add the Resin:
a. Vortex the resin at maximum speed for at least 10 seconds, until it is homogeneous
b. Using a wide-bore pipette tip, add to the reaction plate the volume of the Solution specified below.
<table>
<tr><th>Plate Type and Reaction Volume/Well</th><th>Volume of the Solution/Well (µl)</th></tr>
<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>
</table></td></tr>
<tr><td>4</td><td>Seal, vortex, load and run the plates</td><td>Follow the instructions in “After Pipetting Is Complete” on page 4.</td></tr>
</table>

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.

<table>
<tr><th>STEP</th><th colspan="2">ACTION</th></tr>
<tr><td>1</td><td>Calculate the required volume of the Purification reagents.</td><td>Based on your plate and reaction size, calculate the volume of the Solution and Resin needed.
Note: All volumes below include an additional 10% to account for dead volume in the reagent trough.
For 384-well plate, 5-µl reactions:
<table>
<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>
<tr><td>Solution</td><td>24.75</td><td>9504</td><td></td><td></td></tr>
<tr><td>Resin</td><td>5.5</td><td>2112</td><td></td><td></td></tr>
</table>
For 96-well plate, 10-µl reactions:
<table>
<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>
<tr><td>Solution</td><td>49.5</td><td>4752</td><td></td><td></td></tr>
<tr><td>Resin</td><td>11</td><td>1056</td><td></td><td></td></tr>
</table>
For 96-well plate, 20-µl reactions:</td></tr>
</table>

		<table><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><tr><td>Solution</td><td>99</td><td>9504</td><td></td><td></td></tr><tr><td>Resin</td><td>22</td><td>2112</td><td></td><td></td></tr></table>
2	Combine the Reagents to create the premix	Combine the Solution and Resin: a. Vortex the Resin bottle at maximum speed for the least 10 seconds, until it is homogeneous. b. Using a wide-bore pipette tip or a graduated cylinder, add the appropriate volume of Resin to a clean container. **IMPORTANT!** Avoid pipetting near the surface of the liquid. c. Using a conventional pipette tip or a graduated cylinder, add the appropriate volume of the Solution to the container with the Resin. Make sure there are no particulates in the Solution before pipetting. If particulates are present, heat the Solution to 37℃ and mix to redissolve. Cool to room temperature before using. d. Mix the reagents until homogeneous. **Note:** The premix can be stored in a clean, capped container at 4℃ for up to 5 days.
3	Centrifuge the sequencing reaction plates.	Following 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 5.
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><tr><th>Plate Type and Reaction Volume/Well</th><th>Volume of the Solution/Well (µl)</th></tr><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></table> **IMPORTANT!** Mix the premix as needed to maintain a homogeneous solution. Dispense the premix within 1 minutes of aspiration to avoid separation of the reagents in the pipette tip.
5	Seal, vortex, load, and run the plates	Follow the instructions in “After Pipetting Is Complete” on page 4.

After Pipetting Is Complete

STEP	ACTION

<table>
<tr><td>1</td><td>Seal the reaction plates.</td><td>Seal the plate, using:
■ A heat seal at 160 ℃ for 2 seconds
or
■ MicroAmp Clear Adhesive Films or any other good adhesive films. Verify that each well is sealed.
IMPORTANT! If you are using an ABI 3730 DNA Analyzer and plan to use direct injection, only ABI Heat Seal Film for Sequencing and Fragment Analysis Sample Plates is supported.</td></tr>
<tr><td>2</td><td>Vortex the reaction plates.</td><td>Vortex the reaction plate for 30 minutes using the following conditions:
<table>
<tr><th>Vortexer</th><th>Plate Type</th><th>Speed</th></tr>
<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>
</table>
Note: It is recommended that you pause vortexing after 1 minute to verify that the contents are well mixed.</td></tr>
<tr><td>3</td><td>Centrifuge the reaction plates</td><td>In a swinging-bucket centrifuge, spin the plate at 1000 xg for 2 minutes.</td></tr>
<tr><td>4</td><td>Prepare the plates for the instrument run.</td><td>Place the reaction plate in The CE instrument. (To store and run the plate later, see step 6.)
<table>
<tr><th>Plate Type</th><th>Instrument</th><th>Seal</th><th>Instructions</th></tr>
<tr><td rowspan="3">384-well</td><td rowspan="2">3730/3730xl</td><td>Heat seal</td><td>Place directly in the instrument.</td></tr>
<tr><td>MicroAmp Clear Adhesive Film</td><td>■ Remove the clear adhesive film, replace with a heat seal, and then place in the instrument.
■ Transfer 10 µl of supernatant to a clean plate, cover with a septa mat, place in instrument</td></tr>
<tr><td>3100/3100Avant, 3130/3130xl, 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 rowspan="2">960-well</td><td rowspan="2">3730/3730xl</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>
</table></td></tr>
</table>

			3100/3100Avant or 3130/3130xl	Either	Remove the seal, replace with a septa mat, 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.
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: ■ Room temperature – Plates sealed with heat seal film, adhesive film, or septa for up to 48 hours at room temperature (20 to 25℃). ■ Refrigerated storage – Plates sealed with heat seal film or adhesive film for up to 10 days at 4 ℃ (recommended). ■ Frozen storage – Plates sealed with heat seal film or adhesive film for up to 10 days at -20 ℃			

DNA Quantity Guidelines

DNA sequencing reactions purified with the XT Purification Kit result in high signal strength when analyzed on a DNA sequencer. Therefore, when you prepare sequencing samples for purification with the XT Purification 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 if 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 5000 bp	5 to 40		Bacterial genomic DNA	1,000 to 3,000
>2000 bp	10 to 50			