ALINE PureSEQ™ Kit Protocol

Sanger Capillary Sequencing Dye Terminator Removal Kit

Catalog Numbers: P-3001-5, P-3001-50, P-3001 bulk

Contents

Protocol Manual Revision *2.3*

Please refer to http://www.alinebiosciences.com Support section for updated protocols and MSDS when handling or shipping any chemical hazards. The information provided is subject to change without notice.

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INTRODUCTION

PureSEQ™ utilizes a proprietary magnetic bead-based sequencing purification system for sequencing reaction clean up and BigDye® removal. It is simple, flexible and can be used in any automated liquid handling platforms. It is widely used in high throughput sequencing environment and manual processes.

Aline PureSEQ™ has been optimized for BigDye® and other similar sequencing dye sets.

The PureSEQ™ procedures mainly consist of binding, washing and elution steps, and can be performed directly in the thermal cycling plate without centrifugation or filtration.

SPECIFICATIONS

The amount of PureSEQ™ used per sequencing purification reaction depends on the sequencing reaction volume and plate format. Please refer to the table below to determine the number of cleanups each kit can perform.

Number of reactions in each kit

Plate Format	5 mL (P-3001-5) **(# reactions)**	50 mL (P-3001-50) **(# reactions)**
96 well	500	5000
384 well	1000	10000

MATERIALS

ALINE PureSEQ™
- Store at 4°C upon arrival, for up to 12 months.
- Mix PureSEQ™ well before using. It should appear visually homogenous.
- AVOID FREEZING.

MATERIALS SUPPLIED BY THE USER:
Protocol – PureSEQ™ Revsion 2.3

Magnetic Plate:
For 96 well format: ALINE Biosciences, Cat. No.: MagP-96EB, www.alinebiosciences.com or Ambion http://www.invitrogen.com/site/us/en/home/brands/ambion.html.
For 384 well format: 384 Magnet Plate - MagnaBot® 384 Magnetic Separation Device, Promega Corporation, #V8241, www.promega.com or Beckman Agencourt® SPRIPlate® 384 Magnet Plate, https://www.beckmancoulter.com

Reaction Plate:
For 96 well format: 96 Well Cycling Plate; Suggested ABGene #AB-1000 or AB-1400, http://www.abgene.com/
For 384 well format: 384 Well Hardshell Cycling Plate; Suggested ABGene Diamond #AB-1111, http://www.abgene.com/
- Multichannel pipettes for 10 µL and 100 µL volumes

Reagents:
- 85% Ethanol made from non-denatured ethanol (ACS grade, Sigma-Aldrich or equivalent)
Make 25mL of 85% ethanol per 96 well plate
Note: It is critical to make fresh 85% ethanol within 1-3 days prior to use. This will help to avoid loss of product. Store this ethanol solution in a tightly capped container.
- Elution Buffer: nuclease-free water or 0.01mM EDTA (pH 8.0)
Note: The optimal Elution Buffer will vary depending on dye chemistry and reaction conditions.

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Procedure – ABI BigDye® Terminator Removal 96 Well

1. Gently shake PureSEQ™ bottle to fully re-suspend the magnetic beads before every use.
IMPORTANT: The reagent should appear homogenous and clear in color. No precipitation should be observed. Bottle should be tightly sealed and stored at 4°C after being opened.

2. Add 10 µL of PureSEQ™ to each sample.
Note: Use 10 µL of PureSEQ™ regardless of the sequencing reaction volume.

3. Add 85% ethanol to each sample according the table below. Pipette mix 7 times or until the solution is homogenous.
Note: It is very important to mix the Ethanol and the PureSEQ™ thoroughly for a complete binding of the sequencing products to the magnetic beads.

PureSEQ™ for 96-well BigDye® Terminator Removal	
Sequencing Reaction Volume (µL)	Volume of 85% Ethanol (µL)
5	31
10	42
15	52
20	62
25	73

4. Place the sample plate onto a 96 well magnetic plate for 3 - 5 minutes or until solution is clear.
Note: The magnetic beads will form either a ring or crescent or dot on the side of the well.

5. Remove the cleared solution (supernatant) slowly from the plate by aspiration.
Note: This step must be performed while the plate is situated on the magnet. To avoid disturbing the beads, place the pipette tip at the bottom of the well when aspirating. Remove as much supernatant as possible as it contains excess fluorescent dye as well as contaminants.

6. Dispense 100 µL of 85% ethanol into each well. Wait at least 30 seconds or until the beads have resettled before moving to the next step.
Note: This step should be performed with the plate situated on the magnet. It is not necessary to mix or resuspend the beads during this step.

7. Completely remove the ethanol and discard.
Note: This step must be performed while the plate is on the magnet. When aspirating, place the pipette tip at the bottom of the well to avoid disturbing the beads. Remove as much ethanol as possible to avoid excess fluorescent dye and contaminants carryover.

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8. Repeat the washing steps 6 /7 for one more time with 85% ethanol if 4ul or more of Dye Terminator is used in the sequencing reaction.

9. Let the samples air-dry for 10 minutes at room temperature.
Note: The sample plate can be on or off the magnet while drying. Avoid excessive drying that can lead to degradation of the fluorescent dye.

10. Add 40 µL of Elution Buffer (see chart below) and incubate the plate for 5 minutes at room temperature to elute.
Note: Elution of the sequencing products is rapid. It is not necessary for the beads to go back into solution for complete recovery. Do not denature samples before loading on capillary sequencers.

IMPORTANT: The suggested Elution Buffers are bio-reagent grade water or 0.01mM EDTA (pH 8.0). Water is used for maximum signal, while EDTA is used to lower the signal in cases where the signal is too strong. The appropriate Elution Buffer will vary depending on the sensitivity of the sequencing detector, the amount of BigDye® used per sequencing reaction and the type of template. Use the following table as a general guideline for an Elution Buffer selection. Adjustment may be necessary based on signal strength and noise.

	ABI 3100 / 3130	ABI 3700	ABI 3730
>2 µL BigDye® with PCR Products	0.01mM EDTA	0.01mM EDTA	0.01mM EDTA
<2 µL BigDye® with PCR Products	0.01mM EDTA	DiH2O	0.01mM EDTA
>2 µL BigDye® with Plasmids	Plasmids 0.01mM	DiH2O	0.01mM EDTA
<2 µL BigDye® with Plasmids	Plasmids DiH2O	DiH2O	DiH2O

11. Allow the sample plate to separate on the magnet for 3-5 minutes or until solution is clear. Transfer 35 µL of the clear sample into a new plate for loading on the detector.
Note: Leave 5 uL of liquid behind to avoid transferring beads into the final plate. Residual beads interfere with injection, causing late starts or failed injections. If this occurs, simply re-transfer the samples away from the beads and re-inject.

Samples can be sealed and stored at 4°C for up to 24 hours, prior to loading. Store the samples at -20°C if samples will not be loaded within the 24 hours timeframe. Samples can be kept at -20°C for 3 month.

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Procedure – ABI BigDye® Terminator Removal 384

1. Gently shake PureSEQ™ to fully re-suspend the magnetic beads before every use.
Note: The reagent should appear homogenous and clear in color. No precipitation should be observed.

2. Add 5 µL of PureSEQ™ to each sample. Use 5 µL of PureSEQ™ regardless of the sequencing reaction volume.

3. Add 85% ethanol to each sample according to the table below. Pipette mix 7 times or until the solution is homogenous throughout each well.
Note: It is very important to mix the layers of ethanol and PureSEQ™ well in order to completely bind the sequencing products to the magnetic beads.

PureSEQ™ for 384-well BigDye® Terminator Removal	
Sequencing Reaction Volume (µL)	Volume of 85%Ethanol (µL)
5	14.3
10	21.4
15	28.6

4. Place the sample plate on a 384 well magnetic Plate for 2 - 3 minutes or until the solution is clear.
Note: The magnetic beads will be pulled to the side of the well as dots.

5. Remove the cleared solution (supernatant) from the plate by aspirate.
Note: This step must be performed while the plate is on the magnet. Place the pipette tip at the bottom of each well when aspirating to avoid disturbing the beads. Remove as much supernatant possible as it contains excess fluorescent dye and contaminants.

6. Dispense 30 uL of 85% ethanol into each well then pipette mix 1-3 times to wash the beads. Incubate at least 30 seconds or until the beads have resettled before continuing to the next step.
Note: This step should be performed while the plate is situated on the magnet.

7. Completely remove the ethanol and discard.
Note: This step must be performed with the plate situated on the magnet. Place the pipette tip at the bottom of the well when aspirating to avoid disturbing the beads. Remove as much ethanol as possible to remove excess fluorescent dye and contaminants.

8. Repeat washing steps 6/7 for one more time with 85% ethanol.

9. Let the samples air-dry for 10 minutes at room temperature.

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Note: The sample plate can be situated on or off the magnet while drying. *Avoid excessive drying that can lead to degradation of fluorescent dye.*

10. Add 15 - 30 uL of Elution Buffer (see the chart below) and incubate the plate for 5 minutes at room temperature to elute.

IMPORTANT: Elution of the sequencing products is rapid. It is not necessary to mix or re-suspend the beads for complete recovery. Do not denature samples prior to loading on capillary sequencers.

The suggested Elution Buffers are 0.1mM EDTA (pH 8.0) or reagent grade water. Water is used to give maximum signal, while EDTA is used to lower the signal in cases where the signal is too strong. The appropriate Elution Buffer will vary depending on the sensitivity of the sequencing detector, the amount of BigDye® used per sequencing reaction and the type of template. Use the following table as a general guideline for choosing an Elution Buffer.

	ABI 3100 / 3130	ABI 3700	ABI 3730
>2 µL BigDye® with PCR Products	0.01mM EDTA	0.01mM EDTA	0.01mM EDTA
<2 µL BigDye® with PCR Products	0.01mM EDTA	DiH2O	0.01mM EDTA
>2 µL BigDye® with Plasmids	Plasmids 0.1mM	DiH2O	0.01mM EDTA
<2 µL BigDye® with Plasmids	Plasmids DiH2O	DiH2O	DiH2O

11. Allow the sample plate to separate on the magnet for 3-5 minutes or until solution is clear. Transfer clear sample into a new plate for loading on the detector.
Note: Leave 2 – 5uL of liquid behind to prevent transferring beads into the final plate. Residual beads can interfere with injection, causing late starts or failed injections. If this occurs, simply retransfer the samples away from the beads and re-inject.

Seal samples and store at 4°C, for up to 24 hours, prior to loading. Otherwise store the samples at -20°C if samples will not be loaded within 24 hours. Samples can be kept at -20°C for at least 3 month.

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