ALine DNA Normalizer™ v3 Kit

Catalog Numbers: N-4300-100, N-4300-1000, N-4300-2000

Contents

Protocol Manual *Revision v7.3*

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

ALine DNA Normalizer™ utilizes ALine's patent pending paramagnetic bead technology for PCR DNA, sheared genomic DNA. Our beads have limited binding surface, therefore by limiting the amount of beads added in a given purification reaction, pre-defined amount DNA can be isolated based on customers need. DNA normalization is accomplished during this purification process so that additional DNA quantification and dilution are not necessary. Time, labor and reagent cost are greatly saved with our unique normalization purification system. The protocol mainly consists of binding, washing and elution steps, and can be performed directly in the thermal cycling plate and requires no centrifugation or filtration. The process can be automated with walk away solution for high throughput applications.

Process Overview

1. Bind DNA to magnetic beads
2. Wash DNA with ethanol
3. Wash DNA with ethanol
4. Elute DNA.

For automation process, the protocol may be performed on any major liquid handling instruments.

Kit Specifications

The amount of DNA Normalizer™ used per purification reaction depends on the PCR reaction input and plate format. Please refer to the charts below to determine how many cleanups each kit can perform. The binding and recovery capacity of 20ul DNA Normalizer™ Beads is about 100ng. To increase the binding capacity, increase the volume of Normalizer™ beads per reaction. For example, use 40ul beads for 200ng DNA recovery. For normalization of PCR product, reducing the ratio of beads to sample is not recommended.

Table 1 Reaction Numbers when using ALine DNA Normalizer™

	Small (P/N) N-4300-100	**Medium (P/N)** N-4300-1000
96 Well Format (20 µL V3 Beads)	100	1000
384 Well Format (5 µLV3 beads)	400	4000

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Materials Supplied in the Kit

ALine DNA Normalizer™ V3 Kit
- Store at 4°C upon arrival, for up to 12 months.
- Resuspend PCRClean DX (optional) and DNA Normalizer™ V3 Beads well before using.
- DO NOT FREEZE.

Materials Supplied by the User

Magnetic Plate

For 96 well format: 96 well disc magnetic stand

For 384 well format: 384 Magnet Plate - MagnaBot® 384 Magnetic Separation Device, Promega Corporation, # V8241, www.promega.com or equivalent

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

Reagents

- Reagent grade water as Washing Buffer.
- 80% ethanol
- 10 mM NaOH as Elution Buffer. 10mM NaOH may be used in a three-month period if keep in tightly capped container.
- 20mM TrisHCl, pH 7.5

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DNA NORMALIZATION PROCEDURES

Procedure-96 Well PCR Plate Format

1. Shake Aline DNA Normalizer™ V3 Beads until beads are fully resuspended and appear homogeneous.

2. Prepare normalization Reaction Mix (RM) by mixing 20 uL of DNA Normalizer™-V3 Beads and 45 uL of Bind 2 for each DNA sample.

NOTE: Prepare only enough normalization Reaction Mix and discard the unused. The reagent should appear homogenous and consistent in color.

3. Add 65 ul of normalization Reaction Mix (RM) prepared at STEP 2 into each well (containing 10-20 µL DNA in each well) and pipette mix 5 times to completely mix the solution. DNA in water is preferred.

NOTE: input DNA should be more than 300ng. DNA in water without buffer components is preferred. PCR buffer may be fine depending the specific buffer composition.

4. Incubate the mixed sample at room temperature for 25 minutes.

5. Place the sample plate onto a magnet for 1 minute or until solution is clear. Aspirate the cleared solution (supernatant) from the plate and discard.

NOTE: The magnetic beads will form a dot on the side of the well. Gently pipette up and down the solution once may help the beads pellet formation if beads appear to spread out.

6. Dispense 200 µL of 80% ethanol into each well with plate on the magnet. Do not disturb the beads. Incubate for 30 seconds.

7. Remove the supernatant from the bottom of the well 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.

8. Repeat STEPS 6-7 once. Finally air dry the beads for minutes.

9. (Optional) prepare a fresh plate by adding 10 ul of 20 mM TrisHCl, pH 7.5 into each well for use at step 11.
NOTE: This elution plate is meant to neutralize eluted DNA which is basic. Alternatively, purified DNA product may be neutralized during downstream enzymatic reaction setup.

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10. After removing the plate from the magnet, add 25 µL of elution buffer (10 mM NaOH) and pipette mix the magnetic beads for 15 times or shake the plate at 500rpm to 700rpm for 2 minute at room temperature. Let the plate stand for another 3 minutes before putting plate on magnet.

NOTE: the 3 minute extra incubation time is to ensure DNA release from beads.

11. Replace the plate on magnet. Allow the sample plate to separate on the magnet for 2 minutes or until solution is clear. Transfer 23 µL of the clear sample into a fresh plate or to the elution plate prepared at step 9. Leave 2 uL of liquid behind to prevent transfer of beads into the final plate.

NOTE: 1. DNA stays as double-stranded in 10mM NaOH at room temperature.
2. Neutralization buffer can be added into reaction buffer during PCR or sequencing reaction setup.
3. DNA may be analyzed on an agarose gel without prior neutralization.
4. DNA may be kept at -20°C after neutralization for long term storage.

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