DNA Normalizer™ –v2

Catalog Numbers: **N-4002-S, N-4002-M, N-4002-L**

Contents

Protocol Manual *Revision 17.3*

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™-v2 utilizes ALINE's patent pending paramagnetic bead technology for normalization of genomic DNA, PCR products and plasmids with different concentration. These samples are cleaned up in the normalization process simultaneously. 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. Large quantity of DNA samples can be normalized to the similar concentration, combined in one for applications such as Next Generation Sequencing library preparation. The protocol mainly consists of binding, washing and elution steps. The process 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.

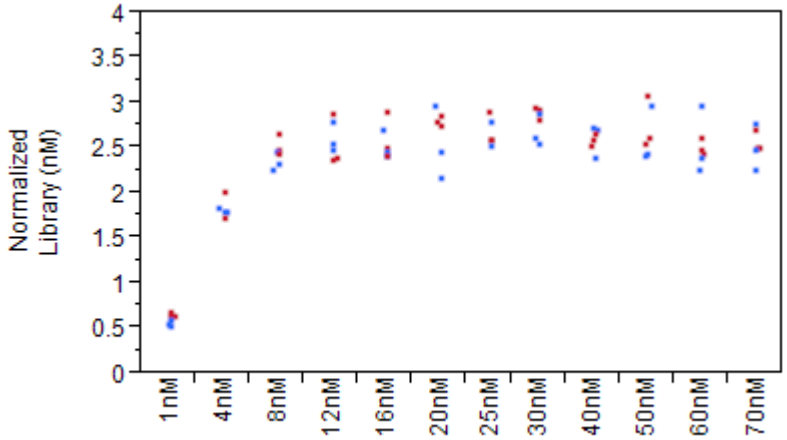

Figure 1. DNA library normalized with DNA Normalizer V2.

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Process Overview

1. DNA bind to magnetic beads, then separate beads on a magnetic plate.
2. Wash beads once to remove contaminants.
3. Elute DNA with Elution buffer.

For automation process, the protocol may be performed on Biomek™, KingFisher™, Hamilton™ and other major automation platforms.

Product Specifications

The amount of DNA Normalizer™-v2 used per purification reaction depends on the PCR reaction input and plate format. Please refer to the chart below to determine the number of samples each kit can perform. The binding and recovery capacity of 20 uL genomic DNA Normalizer™-v2 is about 100 ng. To increase the binding capacity, increase the volume of Normalizer™-v2 beads per reaction. For example, use 40 uL of beads for 200 ng genomic DNA recovery.

Table 1. Number of Reactions Performed using DNA Normalizer™-v2

Desired DNA Yield	N-4002-S (P/N)	N-4002-M (P/N)	N-4002-L (P/N)
96- Well Format (100 ug)	100 reactions	1000 reactions	2000 reactions

Materials Supplied in the Kit

1. ALINE DNA Normalizer™ - Beads - **Store at 4°C** upon arrival (DO NOT FREEZE) for up to 6 months.
   Please Note: Mix DNA Normalizer™ - Beads well by inverting 10 or more times before using.

2. ALINE DNA Normalizer™- Buffer CB2 - **Store at 4°C** upon arrival.
3. ALINE DNA Normalizer™- Buffer EB - Store at room temperature.

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Materials Supplied by the User:

Consumables & Hardware

Magnetic Plate:

For 96-well format: 96- well stand, Ambion Inc., (acquired by Applied Biosystems), #AM10050, www.appliedbiosystems.com or equivalent

Reaction Plate:

For 96-well format: 96-Well Cycling Plate; Suggested ABgene #AB-1000 or AB-1400, http://www.abgene.com/ or equivalent.

- Multichannel pipettes

Reagent:

- 80% ethanol

Procedure – 96 Well Format

1. Shake the Normalizer™-Beads bottle until beads are fully resuspended and appear homogeneous.

2. Prepare normalization Reaction Mix (RM) by mixing 20 uL of DNA Normalizer™-Beads and 80 uL of Buffer CB2 (included in the Kit) for each DNA sample.

NOTE: Prepare only enough normalization Reaction Mix and discard the unused.

Optional: Normalizer™ - Beads and Buffer CB may be added into DNA samples separately.

3. Add 100 uL of normalization Reaction Mix (RM) to 10 uL of each DNA sample. Gently mix by pipetting up and down for five times. Incubate the reaction at room temperature for 25

minutes. (IMPORTANT: Avoid bubble formation by setting the pipette volume to 120 uL. Please make sure NO SHAKING OR VIBRATING of the normalization reaction plate.)

NOTE: A minimum input of 300 ng DNA **(pure DNA in water)** per reaction is required to ensure a final yield of 100 ng DNA. Sufficient or longer incubation time ensures an optimal result when low concentration DNA samples are included.

4. Place the reaction plate on magnetic plate for 3 minute or until beads are settled.

5. Remove supernatant completely and discard.

6. Add 200 uL of 80% ethanol to each well and incubate for 30 seconds. First remove bottom 50 uL solution while the plate is on magnetic plate to remove contaminants.

7. Incubate the plate on magnet for additional 30 seconds and aspirate off the remaining supernatant from the bottom of the well.

8. Dispense 40 µL of Buffer EB (included in the Kit) into each well off magnetic plate. Incubate the reaction plate at room temperature for 3 minutes.

9. Mix the reactions by pipetting up and down for 8 times.

NOTE: To avoid cross contamination, always flash spin the reaction plate if the plate is shaken and before opening the caps for each sample.

10. To collect the normalized DNA

Let the reaction plate sit on magnetic plate for 1 minute or until the solution is clear. Transfer about 38 µL of the clear supernatant into a fresh plate.

Seal samples tightly and store at 4^{o}C for up to 24 hours. For long term storage, store at -20^{o}C.

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If you have any technical questions, please feel free to contact support@alinebiosciences.com or 1-888-987-3677.

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