DNA Normalizer™ –v2

Catalog Numbers: **N-4001-S, N-4001-M, N-4001-L**

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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 patented paramagnetic bead technology for normalization of genomic DNA, PCR products and plasmids with different concentrations. The normalized 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 a 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.

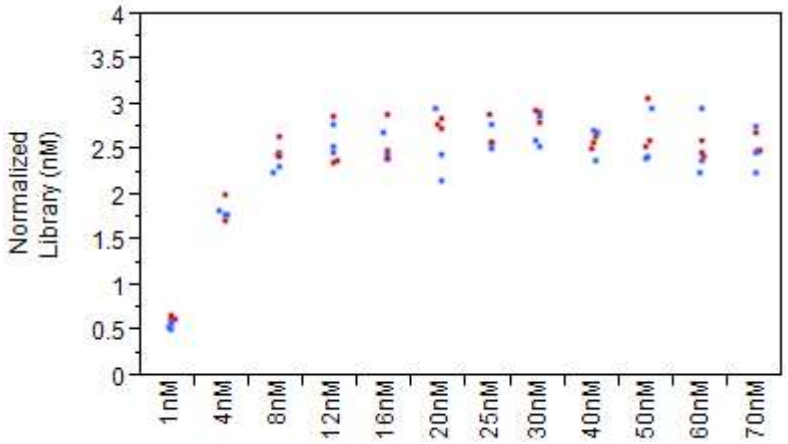

Figure 1. DNA library normalized with DNA Normalizer V2.

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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™, T-Can™, 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-4001-250 (P/N)	N-4001-M (P/N)	N-4001-L (P/N)
96- Well Format (200 ng)	250 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 use.

2. ALINE DNA Normalizer™- Buffer CB （Conditioning Buffer)- **Store at 4°C** upon arrival.
3. ALINE DNA Normalizer™- Buffer EB - Store at room temperature (can also be stored at **4°C** along with other components).

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Materials Supplied by the User:

Consumables & Hardware

Magnetic Plate:

For 96-well format: 24-disc magnetic plate is recommended.

Reaction Plate:

For 96-well format: 96-Well Cycling Plate: BD Falcon Ref 353911, polyvnyl chloride 96 well plate. Www.bd.com

- 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 45 uL of Buffer CB (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.

NOTE: Less beads may added for less DNA yield. More beads may be added for more DNA yield.

3. Add 65 uL of normalization Reaction Mix (RM) to 10-20 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 70

uL during mixing. Please make sure NO SHAKING OR VIBRATING of the normalization reaction plate during incubation period).

NOTE:

(1). **Plate selection is very important.** Different plate may affect yield as well as normalization. If no desired results are produced, switching to different plate may greatly help.

(2). A minimum input of 300 ng DNA **(pure DNA in water)** per reaction is required to ensure a final yield of 200 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 the solution is clear.

5. Remove supernatant completely and discard.

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

7. Incubate the plate on magnet for additional 30 seconds and remove 50 uL liquid from bottom of each well while the plate is on magnetic plate to remove contaminants.

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

9. Dispense 40 µL of Buffer EB (included in the Kit) into each well off magnetic plate. Mix the reactions by pipetting up and down for 5 times.

10. Incubate the reaction plate at room temperature for 5 minutes.

NOTE: DNA binds to the beads tightly. Extended incubation helps DNA to detach from the beads.

11. After incubation, mix the reactions by pipetting up and down for 7 times to fully release DNA.

12. 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°C for up to 24 hours. For long term storage, store at -20°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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