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SPECIFICATION SHEET

NEXTFLEX™ NGS Library Normalization Beads enable fast, on-bead library normalization for NGS library prep. During the final cleanup, the beads bind a fixed mass of library DNA, equalizing molarity across samples so you can skip post-library qPCR or fluorometric quantitation and manual pooling. In high-throughput runs, this saves about three hours per 96-sample batch and supports consistent cluster density for balanced sequencing on any sequencing platform. They can be optimized to work with most NGS library prep workflows.

Part Number	Size
NOVA-NORMBEADS-96	96 reactions

Storage and stability: Store beads at +2-8°C upon receipt. Do not freeze. The shelf life of this reagent is at least 6 months when stored properly.

Usage instructions:

- Bring beads to room temperature before use
- Resuspend beads until homogeneous.
- 100% Ethanol must be added to the NEXTFLEX NGS Library Normalization Beads prior to use. Only prepare enough beads for the number of library preparation reactions.

109 µL	NEXTFLEX NGS Library Normalization Beads
75 µL	100% Ethanol
184 µL	TOTAL (1 reaction)

- Beads saturate at ~100 nanograms of bound library using Revvity's reaction size (184 µL).
- For best performance we recommend using Normalization Beads when yield after PCR is ≥ 250 ng.
- Not recommended for use with PCR-free libraries.

Representative workflow:

Note: The below workflow is taken from Step D of the NEXFLEX Rapid XP v2 DNA-seq kit. The NEXTFLEX NGS Library Normalization Beads can be used with most library preparation protocols but will need to be optimized for each workflow.

1. Add 184 µL of NEXTFLEX NGS Library Normalization Beads to each well containing supernatant from previous step (25 µL). Mix thoroughly until homogenized.
    a. Ensure 100% Ethanol has been added to the NEXTFLEX® Normalization Beads V2. (See Usage instructions)
2. Incubate at room temperature for 8 minutes.
3. Place the 96 well PCR plate on the magnetic stand at room temperature for 5 minutes until the supernatant

appears completely clear.

4. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
5. With plate on stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
6. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.
    a. To aid in ethanol removal, use a clean set of p20 multichannel pipette tips, set the p20 volume to 15 µL and carefully remove any residual ethanol from the bottom of the tube.
7. With plate on magnetic stand, air dry beads at room temperature for 3 minutes.
8. Resuspend dried beads with 23 µL of Elution Buffer.
9. Mix thoroughly until homogenized.
10. Incubate resuspended beads at room temperature for 2 minutes.
11. Place the 96 well PCR plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
12. Do not discard the supernatant in this step. Transfer 20 µL of clear sample to a new well.
13. Remove the 96 well PCR plate from the magnetic stand and discard.
14. Pool 5 µL of each eluted sample into a single Pooled Library tube (1.5 mL tube). Library quantification of pooled material can be performed using fluorometric methods [recommended: Qubit] to determine concentration.
15. The library is now ready for cluster generation per the standard Illumina® protocol. Proceed to cluster generation or seal with adhesive PCR plate seal and store at -20 °C.

Contact us at https://www.revvity.com/contact-us/technical-support using the category “next gen sequencing” for guidance if needed.