MDX224 Lyophilized NGS Enzymatic DNA Fragmentation Kit

Product Manual

1. KIT CONTENTS

Lyophilized NGS Enzymatic DNA Fragmentation Mix	1 strip of 8-wells
Stop Solution	100ul

2. STORAGE

The kit and its components can be stored at ambient temperature (15 - 25°C).

3. SAFETY INFORMATION

When working with chemicals, always wear suitable personal protective equipment, including a lab coat, gloves, and safety glasses.

4. PRODUCT SPECIFICATIONS

The Lyophilized NGS Enzymatic DNA Fragmentation Kit is an enzyme-based reagent that shears DNA to generate fragments of a desired size in a time-dependent manner for next-generation sequencing library preparation protocols.

5. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Thermal cycler or heat block
- Equipment for the determination of DNA concentration, such as Nanodrop™, Qubit™, or equivalent
- Equipment for the determination of DNA size distribution, such as Tapestation™, Bioanalyzer, or equivalent
- Reagents and equipment for the purification and size selection of DNA fragments, such as Meridian NGS Clean and Select Beads or Beckman Coulter AMPure™ XP beads (with magnetic device)
- Molecular-grade water

6. PROTOCOL

The following protocol has been optimized for the fragmentation of 1µg* of DNA.

**** If using different DNA concentration, further protocol optimizations are required (see Table 2).***

IMPORTANT NOTES:

a) If a thermocycler is used, we recommend setting the heated lid "ON" at 85°C.
b) The Lyophilized NGS Enzymatic DNA Fragmentation Mix is very active and starts shearing as soon as the solution containing the DNA template comes in contact with the product. Therefore, we advise setting the thermocycler to 32°C beforehand and placing the program on "hold" until the samples are loaded in step 3.
c) During the set-up, all steps should be performed on ice.

1. Remove the Lyophilized NGS Enzymatic DNA Fragmentation Mix strip from the pouch.
2. Resuspend each lyophilized mix by adding the desired amount of input genomic DNA into a final volume of 20 µL, as described in Table 1.

Table 1. Resuspension of the lyophilized reaction

Reagent	Quantity
Genomic DNA	1ug
Molecular-grade water	up to 20 µL

3. Vortex the tube quickly for several seconds and spin down.

Incubate at 32°C for the recommended times shown below to generate the desired fragment size.

Table 2. Fragmentation reaction conditions

Desired fragment size (bp)	Incubation time (min)**
300 - 500	15-25
600 - 800	10-15

*****To determine the exact incubation time required for a given sample type and/or different input genomic DNA concentration, a time course study should be performed.***

4. Add 5 µL of Stop Solution to stop the reaction. Vortex and spin down briefly.
5. Transfer the reaction tube to ice.
6. Clean up the fragmented DNA using either column purification or magnetic beads (Meridian NGS Clean and Select Beads or Beckman Coulter AMPure™ XP beads (with magnetic device)). If using beads, it is recommended to dilute the sample 1:1 with 25 µL of sterile water for easier handling of the sample and faster collection of the beads to the magnet.

6.2 Post-Fragmentation Clean-Up

1. Allow AMPure XP beads to equilibrate at room temperature for at least 30 mins. Vortex beads thoroughly to ensure beads are homogenously suspended.
2. Perform a 1.8x bead-based clean-up by adding 90 µL of homogenous AMPure XP beads to each DNA sample. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 mins.
3. Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 mins).
4. Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
5. Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 80% freshly prepared ethanol to each tube. **IMPORTANT**: Always use freshly prepared 80% ethanol.
6. Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
7. Repeat wash (steps 5 to 6).
8. After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use P20 or P10 pipettes and tips to aspirate small volumes of residual ethanol.
9. Leave the lids open and dry the beads at room temperature for 3-5 mins or until the residual ethanol has completely evaporated. **IMPORTANT**: Do not over-dry the beads as this will decrease yield. The bead pellet is dry when the appearance of the surface changes from shiny to matte.
10. Remove tube(s)/plate from the magnetic stand. Add **42 µL of Molecular-grade water** to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 5 mins at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 mins or until the solution is clear.
11. Remove 40 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.

NOTE: We recommend performing the clean-up step immediately after fragmentation. However, if the user intends to stop after fragmentation without clean-up, it is suggested to store the fragmented DNA overnight at 4°C. The clean-up step can be continued on the following day without affecting the quality or the yield of the fragments.

Clean-up of the fragmented DNA is recommended for downstream applications; TapeStation, NGS library preparation, PAGE gel, etc, except for an agarose gel. Samples can be loaded directly onto an agarose gel. It is not necessary to clean up the reactions before loading.

Fragmented DNA can be stored at 4°C overnight or for short-term storage; however, clean-up is recommended before long-term storage at -20°C.