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DNA Fragmentation Reagent for ONT

Product description

DNA Fragmentation Reagent for ONT is a library preparation kit specifically designed for third-generation Nanopore sequencing platforms. This kit uses high-quality fragmentation enzymes, eliminating the need for cumbersome ultrasonication processes and simplifying the workflow by combining the fragmentation module with end-repair and dA-tailing modules. It significantly reduces the time and cost of library preparation. Suitable for 50 ng to 1 μg of genomic DNA from common animals, plants, microorganisms, etc., it enables DNA fragmentation, end repair, and A-tailing in a single tube.

Specifications

Cat.No.	N210008E / N210008S / N210008M
Size	8 T / 24 T / 96 T

Components

Components No.	Name	N210008E	N210008S	N210008M
N210008-A	Smearase™ Buffer	80 μL	240 μL	960 μL
N210008-B	Smearase™ Enzyme	40 μL	120 μL	480 μL

Storage

This product should be stored at -25~-15°C for 1 year.

Notes

1. This product is intended for research use only.
2. For safety and health, wear a lab coat and disposable gloves during operation.
3. Purchase the appropriate reagents compatible with the Nanopore platform in advance to facilitate experiments.
4. The kit is compatible with an input range of 50 ng to 1 μg of DNA. Use high-quality DNA with an A260/A280 ratio of 1.8-2.0 whenever possible.
5. For standard high-quality genomic DNA, refer to Table 1 for recommended fragmentation times. The kit has low bias and can tolerate various GC contents.

Table 1: Recommended Fragmentation Times for Standard Genomic DNA

Main Peak Size	Fragmentation Time	Optimization Range
6-8 kb	3 min	2-4 min
3-5 kb	5 min	4-6 min
2-3 kb	8 min	6-9 min
1.5-2 kb	10 min	9-11 min
1-1.5 kb	15 min	12-18 min
1kb	20 min	18-20 min

750 bp	25 min	22-25 min

[Note]: These are recommended times. Users should fine-tune them in their own experimental systems to achieve optimal results.

6. To ensure optimal and consistent fragmentation results, perform the fragmentation process on ice.

Instructions

DNA Fragmentation / End Preparation / dA-Tailing

This step fragments genomic DNA while simultaneously performing end repair and dA-tailing.

1. Thaw the reagents listed in Table 2, mix by inversion, and place them on ice for use.
2. Prepare the reaction mixture on ice according to Table 2.

Table 2: Genomic DNA Fragmentation Reaction System

Component	Volume (μL)
gDNA	X
Smearase™ Buffer	10
Smearase™ Enzyme	5
ddH_2O	Up to 60

3. Gently mix the reaction mixture by pipetting (do not vortex), and briefly centrifuge to collect the liquid at the bottom of the tube.
4. Place the PCR tube into a PCR machine and set the program as shown in Table 3:

Table 3: Genomic DNA Fragmentation Program

Temperature	Time
4°C	1min
30°C	3-25 min*
65°C	20 min
4°C	hold

[Note]:

*To effectively control fragmentation and avoid over-digestion, set the initial temperature to 4°C. Once the block reaches 4°C, insert the PCR tube.

**For intact genomic DNA, refer to Table 1 for recommended digestion times.

5. If purification is required, it is recommended to use NGS DNA Selection Beads (Cat#N210362). Follow the steps below:

I. Preparation: Remove the NGS DNA Selection Beads from the refrigerator and allow them to equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.

II. Vortex or thoroughly invert the magnetic beads before use.

III. For full recovery of all fragments, use a 1.0–1.2× bead-to-sample ratio (i.e., add 60–72 μL of beads to the fragmented sample). If only larger fragments are desired, adjust the bead ratio accordingly. A minimum ratio of 0.3× can be used to recover fragments larger than 4 kb. Mix gently by pipetting 10 times and incubate at room temperature for 10 minutes.

IV. Briefly centrifuge the PCR tube and place it on a magnetic stand. Once the solution becomes clear (approximately 5 minutes), carefully remove the supernatant.

V. Keep the PCR tube on the magnetic stand at all times. Add 200 µL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove the supernatant.

VI. Repeat step V for a total of two washes.

VII. Keep the PCR tube on the magnetic stand. Open the lid and air-dry the beads until no ethanol odor remains (recommended not to exceed 5 minutes).

VIII. Remove the PCR tube from the magnetic stand and add 23 µL of nuclease-free ddH_2O or elution buffer (10 mM Tris-HCl, pH 8.0–8.5) to cover the beads. Mix by pipetting and incubate at 37°C for 10 minutes. Extend incubation time if beads have dried out.

IX. Place the PCR tube on the magnetic stand until the solution clears (approximately 5 minutes).

X. Carefully transfer 21 µL of the supernatant to a new PCR tube and use 1 µL for Qubit quantification.

6. Both purified and unpurified products can be directly used for barcode or adapter ligation using: DNA Library Prep Kit for ONT(Cat#N210007)

Please refer to the respective user manuals for detailed instructions.