Hieff NGS™ OnePot Pro DNA Fragmentation Reagent

Product description

Hieff NGS™ OnePot Pro DNA Fragmentation Reagent is a next-generation enzymatic fragmentation reagent specifically designed for high-throughput sequencing platforms such as Illumina™ and MGI™. This product employs high-quality fragmentation enzymes, eliminating the need for cumbersome sonication procedures. It streamlines the workflow by combining the DNA fragmentation, end repair, and dA-tailing steps into a single reaction, significantly reducing both time and cost in library preparation. It is suitable for input DNA ranging from 500 pg to 1 μg, including standard plant and animal genomic DNA, microbial genomic DNA, and other sample types. The reagent enables DNA fragmentation, end repair, and dA-tailing to be completed in a single tube. The reaction products do not require purification and can be directly used in downstream adapter ligation reactions with Hieff NGS™ Novel DNA Ligation Module (Cat#12626).

Specifications

Cat NO.	12619ES24 / 12619ES96
Size	24 T / 96 T

Components

Components No.	Name	12619ES24	12619ES96
12619-A	Smearase™ Buffer	240 μL	960 μL
12619-B	Smearase™ Enzyme	120 μL	480 μL

Storage

Shipped on dry ice. This product should be stored at -25~-15°C for 18 months.

Notes

1. This kit is compatible with input DNA amounts ranging from 500 pg to 1 μg. High-quality input DNA with an A260/A280 ratio of 1.8–2.0 is recommended.
2. High concentrations of metal ion chelators or other salts in the input DNA may interfere with downstream reactions. It is recommended to dilute DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for fragmentation.

Table 1. Recommended Fragmentation Times for Standard gDNA

Insert Size (Main Peak)	Fragmentation Time	Optimized Range
600 bp	8 min	6–12 min
350 bp	10 min	8–14 min
250 bp	12 min	10–15 min
200 bp	15 min	13–18 min
150 bp	20 min	15–25 min

Table 2. Recommended Fragmentation Times for FFPE DNA

Insert Size (Main Peak)	Fragmentation Time	DIN*
250 bp	9–13 min	> 8.0
250 bp	8–11 min	6.5–8.0
250 bp	4–8 min	4.2–6.5
250 bp	3–6 min	2.5–4.2

[Note]: *DIN (DNA Integrity Number) is a metric defined by the Agilent 2200 system to assess the degradation level of FFPE DNA.

3. For standard high-quality genomic DNA, refer to Table 1 for recommended fragmentation times. The kit exhibits low sequence bias and tolerates templates with various GC contents. For FFPE samples of varying degradation levels, refer to Table 2 for fragmentation time selection. The provided times are recommendations; users should fine-tune conditions within their own experimental systems to achieve optimal results.

4. To achieve the desired DNA fragment size as indicated in the recommended protocols, perform the fragmentation reaction on ice to ensure consistent and high-quality fragmentation.

5. For safety and hygiene, wear a lab coat and disposable gloves during operation.

Instructions

DNA Fragmentation / End Repair / dA-Tailing

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

1. Thaw the reagents listed in Table 3, invert gently to mix, and place on ice until use.
2. Prepare the reaction mixture on ice according to Table 3.

Table 3. Reaction System for DNA Fragmentation / End Repair / dA-Tailing

Reagent	Volume (μL)
Input DNA	x
Smearase™ Buffer	10
Smearase™ Enzyme	5
TE	Up to 60 μL

3. Mix gently by pipetting or vortexing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.
4. Place the PCR tube in a thermal cycler and run the program outlined in Table 4 for DNA fragmentation, end repair, and dA-tailing.

Table 4. Thermal Cycling Program for DNA Fragmentation / End Repair / dA-Tailing

Temperature	Time
Heat Lid 105°C	On
4°C	1 min*
30°C	3–20 min**
65°C	20 min
4°C	Hold

[Note]: *To effectively control fragmentation and prevent over-digestion, pre-cool the thermal cycler block to 4°C before loading the reaction tubes.

**Note: For intact genomic DNA, refer to Table 1 for fragmentation time. Recommended input is 500–1000 ng; the digestion time may be extended by 2–4 minutes based on specific requirements. For FFPE DNA samples of varying quality, refer to Table 2 for appropriate digestion times.

5. If purification is required, use Hieff NGS™ DNA Selection Beads (Cat#12601), or AMPure XP Beads (Cat#A63880), or equivalent products. Alternatively, the reaction product can be directly used in adapter ligation

with Hieff NGS™ Novel DNA Ligation Module (Cat#12626). Refer to the respective product manuals for detailed instructions.

Reference Example

Fragmentation and library preparation of 200 ng Human gDNA (NA12878) using Hieff NGS™ OnePot Pro DNA Fragmentation Reagent. The fragmentation results are shown in Figure 1.

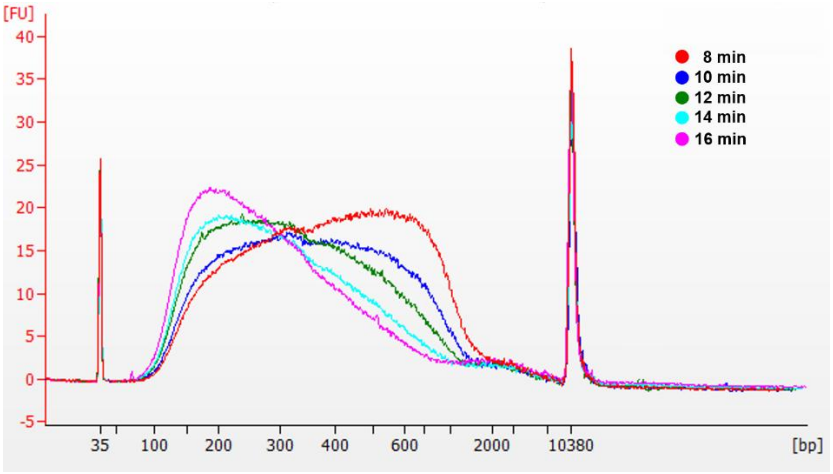


Figure 1. Fragmentation of 200 ng Human gDNA (NA12878) using OnePot Pro DNA Fragmentation Reagent (evaluation of fragmentation efficiency at different digestion times)