

Hieff NGS™ OnePot Pro DNA Fragmentation Module

Product Information

Product Name	Cat#	Specification
Hieff NGS™ OnePot Pro DNA Fragmentation Module	12619ES24	24 T
	12619ES96	96 T

Product Description

Hieff NGS™ OnePot Pro DNA Fragmentation Module provides a new-generation enzymatic fragmentation reagent designed for Illumina® and MGI® high-throughput sequencing platforms. This product simplifies the operation process compared with the complex mechanical interruption and also greatly reduces the time and cost by performing fragmentation, end repair and dA-tailing of dsDNA in one reaction. The fragmentation products can immediately for adaptor ligation with no cleanup steps by using Hieff NGS™ Fast-Pace DNA Ligation Module (Cat#12607) or Hieff NGS™ Novel DNA Ligation Module (Cat#12626). Hieff NGS™ OnePot Pro DNA Fragmentation Module can work with a broad range of DNA inputs (animal, plant and microbial genomes), ranging from 500 pg-1µg in library preparation.

Package Information

Component		12619ES24	12619ES96
12619-A	Smearase™ Buffer	240 µL	960 µL
12619-B	Smearase™ Enzyme	120 µL	480 µL

Shipping and Storage

Hieff NGS™ OnePot Pro DNA Fragmentation Module is shipped with ice pack and can storage at -20°C for 1 year.

Cautions

1. This product is compatible with a range of 500 pg - 1 µg input DNA. High quality input DNA (A260/A280 = 1.8-2.0) is highly recommended.
2. High concentration of metal ion chelator or other salt remained in Input DNA may affect subsequent experiments, it is recommended to dilute DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Table 1. Guideline for choosing the fragmentation time of conventional genomic DNA

Main peak size of the insert fragment	Fragmentation time	Optimization 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. Guideline for choosing the fragmentation time of FFPE DNA

Main peak size of the insert fragment	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
250 bp	9-13 min	> 8.0

Note: *DIN is DNA Integrity Number, which a method to define the degree of FFPE DNA degradation by using Agilent 2200.

3. For conventional high-quality genomic DNA, fragmentation time refers to Table 1. This product is compatible with various samples with different GC content and has minimal bias. For the FFPE samples with different degrees of degradation, the fragmentation time refers to Table 2. Customers need to fine-tune the recommended fragmentation time in their own experimental system to achieve the best results.

4. The DNA can be digested to the required size according to the recommended fragmentation time. In order to ensure the high-quality and stable fragmentation effect, the fragmentation process should be operated on ice.

5. For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.

Instructions

DNA Fragmentation/End Repair/dA-Tailing

This step performs genomic DNA samples fragmentation, end-repair and dA-tailing in one reaction.

1. Thaw the reagents list in Table 3. Invert and mix thoroughly, and place them on ice for later use.
2. Prepare the reaction system on ice according to Table 3 below.

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

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

3. Gently mix by pipetting or shaking, Centrifuge briefly to get the solution down.
4. Place the tube in a thermocycler and set the program according to table 4 to perform DNA fragmentation, end-repair, and dA-tailing reaction.

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

Temperature	Time
Heat lid to 105°C	On
4°C	1 min*
30°C	3-20 min**
65°C	20 min
4°C	Hold

Note: *Pre-set the program to 4°C to effectively control the fragmentation performance and avoid over-fragmentation. Please place the reaction tube into the thermocycler after the heat block is cooled to 4°C.

5. **Please refer to table 1 for the fragmentation of intact genomic DNA. The fragmentation time is recommended to extent by another 2-4 minutes if the input DNA amount is 500-1000 ng. For FFPE DNA samples with different quality, please refer to table 2. For product purification, please use Hieff NGS™ DNA Selection Beads (Cat#12601) or AMPure XP Beads (Cat#A63880) or other equivalents.