HB220412

Hieff NGS™ FFPE DNA Repair Reagent

Product Information

Product Name	Cat#	Specification
Hieff NGS™ FFPE DNA Repair Reagent	12606ES24	24 T
	12606ES96	96 T

Product Description

Hieff NGS™ FFPE DNA Repair Reagent is a repair reagent designed for FFPE samples. This reagent repairs different kinds of damaged in low-quality FFPE samples and improves the library yield and sequencing quality. This reagent is suitable for Yeasen Hieff NGS™ Ultima DNA Library Prep Kit for Illumina® (Cat#12199), which can repair 5 ng - 1 μg fragmented FFPE DNA without extra operation steps. It is recommended to process repairment and purification before library construction when using other brand reagents for library preparation.

Product Components

Components		12606ES24	12606ES96
12606-A	FFPE DNA Repair Mix	120 μL	480 μL
12606-B	FFPE DNA Repair Buffer	144 μL	576 μL

Shipping and Storage

All the components are shipped with dry ice and can be stored at -20°C for one year.

Cautions

1)For your safety and health, please wear lab coats and disposable gloves to operate.
2)Thaw all components of the kit at room temperature before use. After thawing, mix thoroughly upside down several times, centrifuge briefly and put on ice for later use.
3)It is recommended to pipette or gently vortex the mixture before preparing the reaction liquid at each step. Vigorous vortexing may impact the library yield.
4)It is highly recommended to use filtered pipet tips to avoid cross-contamination. Be sure to change pipet tips when processing different samples.
5)It is highly recommended to pre-heat the lid of the thermocycler for each reaction step.
6)Improper operations may very likely cause carry-over contaminations through aerosols, impacting the experiment accuracy. It is highly recommended to divide the experiment environment into the pre-PCR and post-PCR regions, with separate sets of devices and disposables in each area. Perform routine cleaning for each area by wiping the surfaces with 0.5% sodium hypochlorite or 10% bleach.
7)For research use only!

Instructions

Ⅰ Used in combination with the kit Hieff NGS™ Ultima DNA Library Prep Kit for Illumina®(Cat#12199)

1. After thawing the reagents in Table 1, mix well upside down and place on ice for use.

2. Prepare the reaction system as shown in Table 1 **on ice** (4℃)

Table 1 The reaction system for End Repair/ dA-Tailing

Components	Volume(μL)
Fragmented FFPE DNA	x
Endprep Mix	10
FFPE DNA Repair Mix	5
ddH_2O	Up to 60 μL

3. Mix well by gently pipetting up and down or shaking at low speed, and centrifuge the reaction solution briefly to the bottom of the tube.

4. Place the PCR tube on the thermal cycler and perform an end repair/dA-tailing reaction according to the reaction procedure shown in Table 2.

Table 2 The reaction programs for End Repair/dA-Tailing

Temperature	Duration
Hot lid 105°C	On
20°C *	**15 min***
72°C	20 min
4°C	Hold

***Note**:* *When use with the Cat#12199 kit, to get the best repair effect, the temperature and time of the end repair program should be adjusted slightly, refer to this instruction.

5. The product can be directly connected and operated in accordance with the instructions of the library preparation kit.

Ⅱ Used in combination with the kit Hieff NGS™ OnePot II DNA Library Prep Kit for Illumina® (Cat#12204)

1. After thawing the reagents in Table 3, mix them upside down and place on ice for use.

2. Prepare the reaction system as shown in Table 3 **on ice** (4℃)

Table 3 The reaction system for Fragment/End Repair/ dA-Tailing

Components	Volume(μL)
Input DNA	x
Smearase® Mix	10
FFPE DNA Repair Mix	5
ddH_2O	Up to 60 μL

3. Mix well by gently pipetting up and down or shaking at low speed, and centrifuge the reaction solution briefly to the bottom of the tube.

4. Place the PCR tube on the thermal cycler and perform a fragment/end repair /dA tailling reaction according to the reaction procedure shown in Table 4.

Table 4 The reaction programs for Fragment/end repair /dA tailling reaction

Temperature	Duration
Hot lid 105°C	On
4 °C	1 min*
30 °C	3-20 min**
72 °C	20 min
4 °C	Hold

Note: *To effectively control the fragmentation effect and avoid excessive digestion, the reaction program can be set at 4° C in advance, When the module temperature drops to 4° C, put the PCR tube into the thermal cycler.

**For FFPE DNA samples of different quality, refer to Table 5 for digestion time

Table 5 FFPE DNA fragmented time selection table

Main peak size of insert fragments	Fragmented 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 represent DNA Integrity Number, degradation degree of FFPE DNA is defined by Agilent 2200 as shown in Figure 1.

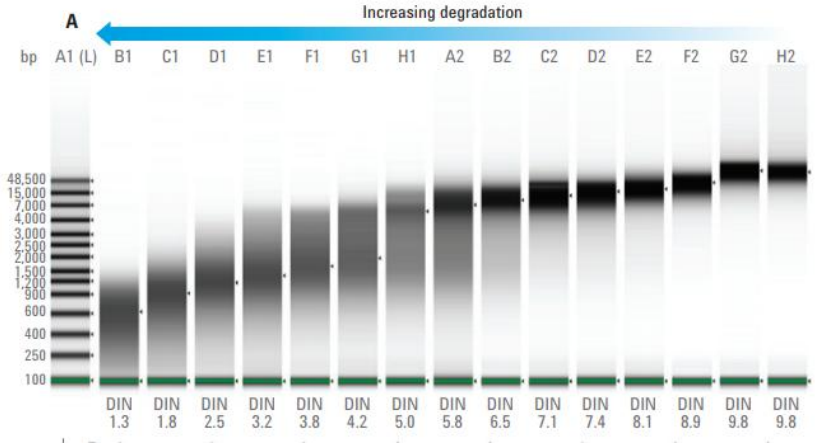

Figure 1 DIN defined different degradation samples by Agilent 2200

Ⅲ Used in combination with other brand reagents (repair and purify before prepare the library)

1. After thawing the reagents in Table 6, mix them upside down and place on ice for use.
2. Prepare the reaction system as shown in Table 6 on ice (4℃).

Table 6 FFPE DNA Repair reaction system

Components	Volume(μL)
Fragmented FFPE DNA	x
FFPE DNA Repair Buffer	6
FFPE DNA Repair Mix	5
ddH_2O	Up to 60 μL

3. Mix well by gently pipetting up and down or shaking at low speed, and centrifuge the reaction solution briefly to the bottom of the tube.
4. Place the PCR tube on the thermal cycler and perform a FFPE DNA repair reaction according to the reaction procedure shown in Table 7.

Table 7 FFPE DNA Repair reaction system

Temperature	Duration
Hot lid 105°C	On
20°C	**15 min**
4°C	Hold

6. Immediately add 108 μL (1.8×) magnetic Beads (Hieff NGS™ DNA Selection Beads, Cat#12601 or AMPure XP Beads, Cat#A63880 or other equivalent products) into the repair products for purification.
7. Process library preparation with the purified product according kit instructions.