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Onestep DNA Frag And ER Reagent

Product Number: FER46

Shipping and Storage

Store at -20℃ and transport on dry ice.

Components

Component	FER46	FER46
FER Buffer	240μl	960μl
FER Enzyme Mix	120μl	480μl

Description

Onestep DNA Frag And ER Reagent is a new generation of enzyme digestion repair reagents developed for Illumina and MGI high-throughput sequencing platforms. It can complete one-step fragmentation/final repair/addition of A. This product includes FER Enzyme Mix and FER Buffer, which can achieve fragmentation and end repair in one tube, avoiding cumbersome ultrasound processes and instrument dependence, and is easy to operate. It can achieve efficient and fast fragmentation, end repair, and dA tail addition for different DNA samples, and is applied to downstream library construction. It has the advantages of low preference, stable enzyme digestion repair effect, and higher library transformation efficiency. It can be applied to fragment, end repair, and A addition reactions of samples from different sources such as 0.1 ng-1ug genomic DNA, PCR amplification products, FFPE, etc.

Protocol

1. Melt the FER Enzyme Mix and FER Buffer, gently flick with your fingers and mix well. Centrifuge briefly to collect and place on ice.
2. Add the following reagents to a 200uL PCR tube (prepare the system on ice and react immediately after preparation):

Component	Volume
Double-stranded DNA	1 ng-500ng
FER Buffer	10μL
FER Enzyme Mix	5μL
NF Water	Up to 50μL
Total	50μL

Note: If DNA is dissolved in a solution containing EDTA or the final concentration of EDTA in the reaction system is ≥ 0.2mM, it is recommended to use magnetic beads for purification before use.

3. Mix gently, centrifuge briefly and place on ice for immediate PCR reaction.
4. Interrupt repair program as shown in the table below (PCR instrument hot cap temperature 70 ℃)

Step	Temperature	Time
1	4℃	1 min
2	32℃	5-30min(Adjustable)
3	65℃	30 min
4	4℃	Hold

The fragmentation time is adjusted according to the size of the target fragment, as shown in the table below.

The relationship between fragmentation time and expected insertion fragment size

	32 ℃ incubation time (min)			
Insert Size	100-200bp	200-300bp	300-400bp	400-500bp
100ng DNA	20-30 min	15-20 min	10-15 min	5-10 min

Note: 1) Select the incubation time at 32 ℃ based on the expected insertion fragment size, and the fragment size will decrease with the extension of reaction time.

2) If there is a slight deviation between the results and the expected fragment size, the reaction time can be adjusted as appropriate, and 3-5 minutes can be added or subtracted from the recommended reaction time.

3) Reduce the fragmentation time of FFPE and other degraded samples appropriately based on their quality.

4) Fragmented repair enzymes are sensitive to time, and it is recommended to precisely control the reaction time after determining the conditions.

5) After this step is completed, LG0N is used for connector connection without purification. When used with other brands of ligases, the compatibility of the system buffer needs to be tested.