VAHTS® Universal Plus Fragmentation,End Preparation & dA-Tailing Module for Illumina

N209

Version 21.1

Product Description

VAHTS Universal Plus Fragmentation, End Preparation & dA-Tailing Module for Illumina is specially designed for Illumina platforms. This kit combines DNA fragmentation, end repair and dA tailing into one step. It is suitable for library preparation from 100 pg - 1 μg of input DNA. With this kit, the input DNA amount is significantly decreased. No mechanical fragmentation of the genome simplifies the process of library construction and shortens the operation time. It is also compatible with automatic library preparation equipment. All the components are subjected to stringent quality control and functional test, ensuring the consistency and reproducibility of library preparation.

Components

Components	N209-01 (24 rxns)	N209-02 (96 rxns)	
FEA Buffer	120 μl	480 μl	■
FEA Enzyme Mix	240 μl	960 μl	■
Neutralization Buffer	120 μl	480 μl	□

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

This kit is applicable to DNA fragmentation, end repair, 5' phosporylation and 3' dA tailing. And it is compatible with 100 pg - 1 μg of input DNA and various kinds of DNA template including genomic DNA (from different species such as animals, plants, and microorganisms), FFPE DNA, etc.

Self-prepared Materials

VAHTS Universal Adapter Ligation Module for Illumina (Vazyme #N204)
VAHTS DNA Adapters (Vazyme #N801/N802,#N805/806/807/808,#N321/N322)
VAHTS DNA Clean Beads (Vazyme #N411)
VAHTS HiFi Amplification Mix (Vazyme #N616)

Notes

1. Thaw all the components at room temperature before use. Mix thoroughly and centrifuge briefly. Put them on ice.
2. The size and distribution of the DNA fragmentation products are determined by a time-dependent enzymatic reaction, so the fragmentation reaction should be prepared on ice.
3. FEA Enzyme Mix is sensitive to EDTA. Please confirm the EDTA concentration of the sample. If the final concentration of EDTA in the fragmentation system is more than 0.1 mM, please pre-treat the sample as the following experiment process.

Experiment Process

Starting material: 100 pg - 1 μg DNA template

1. Before starting the experiment, please confirm which solvent the template DNA is dissolved in (ddH_2O is recommended), whether the solvent contains EDTA. If it does not contain EDTA, proceed directly to **Step 3**; if it contains EDTA, the sample is pre-treated according to **Step 2**.
2. If the solvent contains EDTA, the template DNA can be purified using 2.2 × magnetic beads and eluted by ddH_2O. Alternatively, EDTA can be neutralized by adding corresponding volume of Neutralization Buffer according to the final concentration of EDTA in the fragmentation system.

   ▲ Final concentration of EDTA in fragmentation system = Concentration of EDTA in DNA solution × DNA usage volume/50 μl; e.g., DNA is dissolved in TE buffer containing 1 mM EDTA, and 10 μl is used in the reaction. The final concentration of EDTA is 1 mM × 10 μl/50 μl = 0.2 mM.

Refer to the following table for the volume of Neutralization Buffer:

Final concentration of EDTA in fragmentation system	Volume of Neutralization Buffer
1 mM	5 μl
0.8 mM	4 μl
0.6 mM	3 μl
0.5 mM	2.5 μl
0.4 mM	2 μl
0.2 mM	1 μl
0.1 mM	0.5 μl
<0.1 mM	0 μl

3. Thaw the FEA Buffer and FEA Enzyme Mix. Mix thoroughly and centrifuge briefly. Place on ice until use. All the following steps are performed on ice.

4. Prepare the following solution in a sterile PCR tube:

Components	Volume	
Input DNA	x μl	
Neutralization Buffer	y μl	☐
FEA Buffer	5 μl	■
ddH_2O	To 40 μl	

5. Add 10 μl of FEA Enzyme Mix to each sample, pipetting up and down or vortexing to mix, and centrifuge to collect the reaction solution at the bottom of the tube and **place it in the PCR instrument immediately!**

▲ The fragmentation reaction is a time-dependent enzymatic reaction, and the size of the fragmented products depend on the reaction time. Therefore, it is recommended to add FEA Enzyme Mix to the reaction system separately at last, and mix and perform the subsequent reaction immediately.

▲ The fragmentation reaction system is sensitive to oxidation. Therefore, the lid of FEA Buffer and FEA Enzyme Mix should be tightened as soon as possible after the reaction system is prepared and stored at -20˚C.

6. Put the tube in a PCR instrument and run the following PCR program:

Temperature	Time
Hot lid 105˚C	On
37˚C	Refer to the following table*
65˚C	30 min
4˚C	Hold

* Fragmentation time depends on the quality of input DNA and the expected insert size:

▲ If dA tailing is not performed, 65˚C 30 min can be omitted.

Expected-insert size	Fragmentation time
150 bp	20 - 30 min
250 bp	15 - 20 min
350 bp	10 - 15 min
550 bp	6 - 10 min

▲ The above recommended time was validated using high-quality human placenta gDNA as a template. When using high-quality human placenta gDNA for library construction, within the recommended input range (100 pg - 1 μg), the different input DNA has the same fragmentation time, and the distribution range of fragmentation products is not much different (the distribution range is basically the same, but the main peak position may be slightly different).

If the input DNA is of poor quality or the fragmentation size is not in the expected range, it is recommended to adjust the fragmentation time (± 2 - 5 min). For FFPE DNA, the fragmentation time should be reduced accordingly, depending on its integrity. Immediately proceed to the subsequent reaction after fragmentation.

7. Use VAHTS Universal Adapter Ligation Module for Illumina (Vazyme #N204) for subsequent adapter ligation immediately, or purified by 2 × magnetic beads.