VAHTS Repair Module for ONT V2

TN2025

Version 25.1

Product Description

VAHTS Repair Module for ONT V2 is a module specifically optimized for the Nanopore sequencing platform, suitable for damage repair and end preparation of genomic DNA (gDNA) and amplicons. This upgraded module features a specifically optimized combination of high-activity mutant enzymes paired with a finely tuned buffer system, delivering higher repair efficiency and more robust library preparation performance. All reagents included in this module have undergone rigorous quality control and functional testing to ensure optimal stability and reproducibility of library preparation.

Components

	Components	TN2025-01 (24 rxns)	TN2025-02 (96 rxns)
■	End Prep Buffer	84 μl	336 μl
■	DNA Repair Buffer	84 μl	336 μl
■	End Prep Enzyme Mix	72 μl	288 μl
□	DNA Repair Mix	48 μl	192 μl

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

This module is designed to repair DNA ends and internal DNA damage in input DNA. The repaired products carry a 5' phosphate and a 3' dA overhangs, enabling efficient library preparation for the Nanopore sequencing platform.

Self-prepared Materials

DNA Fragmentation: gDNA Shearing Tube (Vazyme #TDS01501);

DNA Quantification: Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121);

Barcode Ligation: VAHTS BD Ligation Module for ONT V2 (Vazyme #TN2026);

Motor Protein Ligation: VAHTS MP Ligation Module for ONT V2 (Vazyme #TN2027);

Barcode Adapter:

VAHTS TGS DNA BD Adapters Set 1 for ONT (Vazyme #TA20101) or

VAHTS TGS DNA BD Adapters Set 1 - Set 4 for ONT (Plate) (Vazyme #TAB20105);

▲ #TA20101 is supplied in tube format and contains 24 barcodes. #TAB20105 is supplied in plate format and contains 96 barcodes, and is compatible with high-throughput liquid handling workstation (Vazyme #VNL-96P).

3rd party materials (including motor protein adapters and nucleic acid purification buffers):

Ligation Sequencing Kit V14 (Nanopore #SQK-LSK114)

or Native Barcoding Auxiliary Kit V14 (Nanopore #EXP-NBA114);

▲ Motor protein adapters included in #SQK-LSK114 are designed for A/T ligation and single-sample library preparation, whereas those in #EXP-NBA114 are intended for sticky-end ligation and multiplexed library preparation, as barcode ligation generates sticky-end products.

▲ If #EXP-NBA114 is used, Elution Buffer (EB) is required for the elution step in purification of motor protein adapter-ligated products. The EB provided in Sequencing Auxiliary Vials V14 (Nanopore #EXP-AUX003) is recommended.

Clean Beads: VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880);

Other Materials: freshly prepared 80% ethanol, Nuclease-free ddH_2O, low-absorption EP tubes, PCR tubes, magnetic rack, PCR instrument, vortex mixer, etc.

Experiment Process

This step is performed to repair DNA damage, blunt DNA ends, phosphorylate the 5' ends, and add a dA tail to the 3' ends.

1. Thaw End Prep Buffer and DNA Repair Buffer and mix well by inversion. Mix End Prep Enzyme Mix and DNA Repair Mix well by inversion. Keep the reagents on ice. Prepare the reaction solution in a sterile PCR tube on ice as follows:

Components	Volume	
Input DNA*	x μl	
End Prep Buffer	3.5 μl	■
DNA Repair Buffer	3.5 μl	■
End Prep Enzyme Mix	3 μl	■
DNA Repair Mix	2 μl	□
ddH_2O	To 60 μl	

* Recommended Input DNA amount under the current setup:
Genomic DNA (gDNA): For >4 barcodes: 400 ng of input DNA per sample;
For ≤4 barcodes: 1 μg per sample.
PCR-amplified products: ≥200 fmol per sample.

2. Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.

3. Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	5 min
65°C	5 min
4°C	Hold

4. Clean up: VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) is recommended.

a. Equilibrate the beads to room temperature and mix well by vortexing.

b. Add 60 μl of the resuspended beads (1 ×) to 60 μl of the product from the previous step, and gently tap the tube to mix.

c. Incubate at room temperature for 5 min.

d. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off the supernatant.

e. Keep the tube on the magnetic rack and wash the beads with 200 μl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec. Keep the tube on the magnet and carefully pipette off the supernatant.

f. Repeat step e (wash twice in total).

g. Keep the PCR tube on the magnetic rack, and air-dry the beads until there is no residual ethanol.

▲ AMPure XP Beads: Air-dry for 30 sec at room temperature.
▲ VAHTS DNA Clean Beads: Air-dry for 2 - 5 min at room temperature.
▲ The beads are sufficiently dry once the pellet loses its shine. Avoid over-drying, as this will negatively impact DNA recovery.
▲ Drying time may vary depending on the volume of beads used, as well as ambient temperature and humidity conditions.

h. Remove the tube from the magnetic rack and add 25 μl of Nuclease-free ddH_2O to resuspend the beads. Gently tap the tube to mix, and incubate at room temperature. Extend the incubation time as needed when the beads are dry or cracked.

▲ The volume of ddH_2O depends on the downstream experimental steps. Use 25 μl for barcode ligation. For direct adapter ligation (without barcoding), resuspend the beads in 32 μl of Nuclease-free ddH_2O.

i. Place the tube on the magnetic rack until the solution is clear (~ 5 min).

j. Transfer 22.5 μl of the supernatant to a new PCR tube.

▲ If using 32 μl of Nuclease-free ddH_2O for resuspension, transfer 30 μl of the supernatant to a new tube.
▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 3 h to ensure optimal library preparation performance.

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