VAHTS BD Ligation Module for ONT V2

TN2026

Version 25.1

Product Description

VAHTS BD Ligation Module for ONT V2 is a barcode ligation module specifically optimized for the Nanopore sequencing platform. This upgraded module features a specifically optimized combination of high-activity mutant enzymes paired with a finely tuned buffer system, delivering higher ligation efficiency and more robust library preparation performance. All reagents included in this module have undergone rigorous quality control and functional validation to ensure optimal stability and reproducibility of library preparation.

Components

Components	TN2026-01 (24 rxns)	TN2026-02 (96 rxns)
Barcode Rapid Ligase Mix	600 µl	4 × 600 µl

Storage

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

Applications

It is designed to ligate Barcode Adapters with a dT overhang to the ends of repaired products. VAHTS Repair Module for ONT V2 (Vazyme #TN2025) is recommended for the repair step.

Self-prepared Materials

DNA Fragmentation: gDNA Shearing Tube (Vazyme #TDS01501);
DNA Quantification: Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121);
DNA Damage Repair & End Preparation: VAHTS Repair Module for ONT V2 (Vazyme #TN2025);
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 ligate the Barcode to both ends of the repaired products.

1. Mix Barcode Rapid Ligase Mix well by inversion and keep it on ice for later use. Prepare the reaction solution in a sterile PCR tube on ice as follows:

Components	Volume
Purified end-repaired product	22.5 μl
Barcode Adapter*	2.5 μl
Barcode Rapid Ligase Mix	25 μl
Total	50 μl

* Vazyme #TA20101 (tube format, 24 barcodes) or Vazyme #TAB20105 (plate format, 96 barcodes) is recommended as the Barcode Adapter.

▲ Add the Barcode Adapter first, followed by the Barcode Rapid Ligase Mix, to prevent self-ligation.

2. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.

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

Temperature	Time
Heated lid 105°C	On
20°C	20 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 20 μl of the resuspended beads (0.4 ×) to 50 μl of the product from the previous step, and gently tap the tube to mix.

   c. Incubate at room temperature for 10 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 33 μl of Nuclease-free ddH_2O to resuspend the beads. Gently tap the tube to mix, and incubate at 37°C. Gently tap the tube every 2 min to facilitate elution.
   - ▲ For motor protein adapter ligation of pooled samples, where the input volume of barcode ligation products is limited, the elution volume of Nuclease-free ddH_2O per sample may be appropriately reduced (≥12 μl to ensure elution efficiency) to maintain nucleic acid utilization.

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

   j. Transfer 31 μl of the supernatant to a new PCR tube and use 1 μl for Qubit quantification.
   - ▲ When pooling multiple samples with similar DNA fragment sizes, equimolar pooling is recommended. Adjust the total amount of barcode-ligated products to 500 ng - 1.2 μg for subsequent motor protein adapter ligation.
   - ▲ The purified product can be used directly for downstream motor protein adapter ligation or stored at 4°C or -20°C overnight.