VAHTS MP Ligation Module for ONT V2

TN2027

Version 25.1

Product Description

VAHTS MP Ligation Module for ONT V2 is a motor protein adapter 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, enabling efficient ligation of motor protein adapters to both ends of sequencing library fragments. 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	TN2027-01 (24 rxns)
MP Adapter Ligation Buffer	240 µl
MP Adapter Ligase	120 µl

Storage

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

Applications

It is designed to ligate motor protein adapters to both ends of the sequencing library. It is compatible with end-repaired DNA (Vazyme #TN2025) or barcode adapter-ligated DNA (Vazyme #TN2026).

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);
Barcode Ligation: VAHTS BD Ligation Module for ONT V2 (Vazyme #TN2026);
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 motor protein adapters to end-repaired, dA-tailed DNA fragments or barcode adapter-ligated DNA fragments.

1. Thaw MP Adapter Ligation Buffer and mix well by inversion. Mix MP Adapter Ligase well by inversion. Keep them on ice for later use. Prepare the reaction solution in a PCR tube on ice as follows:

Components	Volume
Purified, barcode adapter-ligated DNA or Purified end-repaired product	30 µl
Motor Protein Adapter*	5 µl
MP Adapter Ligation Buffer	10 µl
MP Adapter Ligase	5 µl
Total	50 µl

* Motor Protein Adapter:
▲ Use Nanopore #EXP-LSK114 for single-sample sequencing on one flow cell.
▲ Use Nanopore #EXP-NBA114 for multiplexed barcode sequencing of multiple samples.
▲ Add Motor Protein Adapter first, followed by MP Adapter Ligation Buffer, and finally add MP Adapter Ligase, to prevent self-ligation. For scaled-up reactions, keep the Motor Protein Adapter amount unchanged and scale the remaining components accordingly.

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	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.
▲ For scaled-up reactions, adjust the amount of 0.4 × beads accordingly.

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. Remove the tube from the magnetic rack and add 125 µl of Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) to resuspend the beads. **Do not use 80% ethanol**. Place the tube on a magnetic rack until the solution is clear (~5 min). Keep the tube on the magnet and carefully pipette off the supernatant.
▲ To enrich for DNA fragments of 3 kb or longer, LFB is recommended.
▲ To retain DNA fragments of all sizes, SFB is recommended.

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

g. Keep the PCR tube on the magnetic rack, and air-dry the beads.
▲ 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 15 µl of Elution Buffer (EB) to resuspend the beads. Gently tap the tube to mix, and incubate at 37°C for 10 min. Gently tap the tube every 2 min to facilitate elution.
▲ The EB provided in Nanopore #SQK-LSK114 or #EXP-AUX003 is recommended.

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

j. Transfer 13 µl of the supernatant to a new PCR tube for the subsequent reaction.

k. Refer to the table below to prepare the appropriate library amount based on library size. Library loading volumes may vary between different sequencing platforms and can be adjusted accordingly.

Fragment library length	Flow cell loading amount
Short fragments (<1 kb)	100 fmol
Medium fragments (1 - 10 kb)	35 - 50 fmol
Long fragments (>10 kb)	300 ng

▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 5 days to ensure optimal sequencing results.
▲ Approximate Library DNA molar amount (fmol):
Library DNA (fmol) ≈ (Library DNA (ng) × 1,000) / (0.66 × Average library length (kb))