

HieffTM DNA Library Prep Kit for ONT

13301ES

INSTRUCTIONS FOR USE

Ver. EN20260203

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Product description

This kit is specifically designed for DNA library preparation on third-generation Oxford Nanopore Technologies (ONT) platforms. Through targeted engineering and optimization of enzymes and buffers, it significantly enhances adapter ligation efficiency for long DNA fragments. Moreover, no purification is required after the end repair and dA-tailing steps—samples can proceed directly to barcode ligation, resulting in a faster and more streamlined workflow.

- Input flexibility: Compatible with 100 ng – 1.5 µg of genomic DNA (gDNA) or amplicons
- Rapid protocol: Library preparation completed in approximately 2 hours
- Consistent performance: Rigorous batch-to-batch quality control ensures high reproducibility and stability

Specifications

Cat.No.	13301ES08 / 13301ES24 / 13301ES96
Size	8 T / 24 T / 96 T

Components

Components No.	Name	13301ES08	13301ES24	13301ES96
13301-A	 Endprep Buffer	56 µL	168 µL	672 µL
13301-B	 Endprep Enzyme	24 µL	72 µL	288 µL
13301-C	 Rapid Ligase Master Mix	280 µL	840 µL	4×840 µL
13301-D	 Rapid Ligation Reaction Buffer(5×)	160 µL	480 µL	2×960 µL
13301-E	 Rapid T4 DNA Ligase	40 µL	120 µL	480 µL
13301-F	 Elution Buffer	160 µL	480 µL	2×960 µL

Storage

This product should be stored at -25~-15°C for 1 year.

Notes

I. Operation Guidelines

1. For your safety and health, please wear a laboratory coat and disposable gloves during operation.
2. Before use, thaw all components of the kit on ice. After thawing, mix thoroughly by gently inverting several times. Briefly centrifuge and keep the reagents on ice until use.
3. When preparing reaction mixtures at each step, it is recommended to mix by gentle pipetting. Vigorous vortexing may lead to reduced library yield.
4. To avoid cross-contamination between samples, it is recommended to use filter tips. Always change tips when handling different samples.
5. It is recommended to perform all reactions in a PCR thermocycler equipped with a heated lid. Preheat the thermocycler to a temperature close to the reaction temperature before use.

6. Reagents from different kit lot numbers should not be mixed.
7. This product is for research use only (RUO).

II. Precautions

1. The purification steps after Native Barcode ligation and after Native Adapter ligation require different wash buffers. Please pay close attention to using the correct wash reagent for each purification step.
2. To ensure successful library construction, it is recommended to verify the fragment length and integrity of the input DNA (e.g., by capillary electrophoresis or other equivalent methods).

DNA quality (integrity and fragmentation), purity (e.g., chemical contaminants, residual RNA), as well as excessive or insufficient DNA input may all affect library yield.

3. Please ensure that all required user-supplied materials are prepared in advance to avoid failure to perform the experiment according to the instructions, which may result in sample or reagent loss.
4. When preparing libraries for multiple samples, please record the Native Barcode number used for each sample.

III. Instructions for Use

1. User-Supplied Materials

1)Sample fragmentation: g-TUBE fragmentation, Megaruptor® 3 System (Diagenode), or other equivalent fragmentation methods;

2)DNA quantification: 1× dsDNA HS Assay Kit (Cat. #12642ES); Qubit assay tubes (Cat. #83509ES); or equivalent products from other manufacturers;

3)DNA quality control: TapeStation DNA ScreenTape or other equivalent instruments and systems;

4)Barcode adapters:

Native Barcoding Expansion 1–12 (Nanopore, #EXP-NBD104);

Native Barcoding Expansion 13–24 (Nanopore, #EXP-NBD114);

Native Barcoding Expansion 1–96 (Nanopore, #EXP-NBD196);

Motor protein adapters:

5)Adapter Mix II Expansion (Nanopore, #EXP-AMII001);

Native Adapter (Nanopore, #EXP-NBA114);

6)Purification magnetic beads:

Hieff NGS™ DNA Selection Beads V2 (Cat. #12418); AMPure XP Beads (Beckman); or equivalent products from other manufacturers;

7)Nucleic acid purification buffers:

Additional Long Fragment Buffer (LFB) (Nanopore, #EXP-LFB001);

Additional Short Fragment Buffer (SFB) (Nanopore, #EXP-SFB001);

8)Other materials: Freshly prepared 80% ethanol; Nuclease-free ddH₂O; TE Buffer (10 mM Tris-HCl, pH 8.0–8.5, 1 mM EDTA); Low-binding microcentrifuge tubes; PCR tubes; Wide-bore pipette tips; Magnetic rack; PCR thermocycler; Vortex mixer.

IV. Workflow Overview

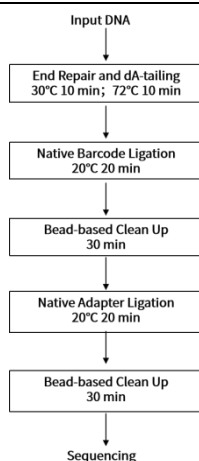


Figure 1. The workflow of DNA Library Prep Kit

Instructions

I. End Repair / dA-Tailing

This step repairs DNA ends, phosphorylates the 5' ends, and adds a single dA overhang to the 3' ends.

1. Thaw Endprep Buffer and Endprep Enzyme on ice. Gently invert to mix, briefly centrifuge, and keep on ice. In a PCR tube, prepare the reaction mixture according to the volumes listed in Table 1 below:

Table 1. End Repair / dA-Tailing PCR Reaction Setup

Components	Volume (μL)
Input DNA	x
Endprep Buffer	7
Endprep Enzyme	3
ddH ₂ O	Up to 60

2. Gently pipette up and down to mix (do not vortex), then briefly centrifuge to collect the reaction mix at the bottom of the tube.

3. Place the PCR tube in a thermal cycler and run the program as specified in Table 2:

Table 2. End Repair / dA-Tailing PCR Cycling Program

Temperature	Time
Hot lid to 105°C	On
30°C	10 min
72°C	10 min
4°C	Hold

II. Native Barcode Ligation

This step ligates Native Barcodes to both ends of the DNA fragments from Section I.

1. Thaw Native Barcode and Rapid Ligase Master Mix on ice. Flick gently to mix, briefly centrifuge, and keep on ice. In a PCR tube, prepare the reaction mixture according to Table 3:

Table 3. Native Barcode Ligation PCR Reaction Setup

Components	Volume(μL)
End-repair&dA-tailing product	60
Native Barcode*	2.5
Rapid Ligase Master Mix	35
ddH ₂ O	Up to 100

[Note]: Native Barcodes are recommended to be Oxford Nanopore Technologies (ONT) official products (e.g., #EXP-NBD104 or #EXP-NBD114).

2. Gently pipette up and down to mix (do not vortex), then briefly centrifuge to collect the liquid at the bottom.
3. Place the PCR tube in a thermal cycler and run the program as specified in Table 4:

Table 4. Native Barcode Ligation PCR Cycling Program

Temperature	Time
Hot lid to 105°C	Off
20°C	20 min
4°C	Hold

III. Purification of Native Barcode Ligation Products

This step purifies the ligation products from Section II using Hieff NGS™ DNA Selection Beads V2 (Cat# 12418).

1. Preparation: Remove magnetic beads from storage and equilibrate at room temperature for 30 min. Prepare fresh 80% ethanol.
2. Vortex or thoroughly invert the bead suspension before use to ensure homogeneity.
3. Add 40 μL Hieff NGS™ DNA Selection Beads V2 (0.4× ratio; Beads:DNA = 0.4:1) directly to the ligation reaction from Section 3.2. Pipette up and down 10 times to mix thoroughly. Incubate at room temperature for 10 min.
4. Briefly centrifuge the PCR tube and place it on a magnetic rack. Wait until the supernatant is clear (~5 min), then carefully remove and discard the supernatant.
5. While keeping the tube on the magnet, add 200 μL freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 sec, then carefully remove the supernatant.
6. Repeat Step 5 for a total of two washes.
7. Keep the tube on the magnet and air-dry the beads with the lid open until no ethanol residue remains (do not exceed 5 min).
8. Remove the tube from the magnet and add 33 μL nuclease-free ddH₂O or elution buffer (10 mM Tris-HCl, pH 8.0–8.5) to cover the beads. Pipette to mix. Incubate at 37°C for 10 min. If beads appear cracked due to over-drying, extend incubation time as needed.

[Note]: If multiplexing multiple samples for subsequent Native Adapter ligation, consider reducing the elution volume accordingly.

9. Place the tube back on the magnet and wait until the supernatant is clear (~5 min).
10. Transfer 31 μL of the supernatant to a new PCR tube. Use 1 μL for Qubit quantification.

IV. Native Adapter Ligation

1. This step ligates Native Adapters to the purified, barcoded DNA from Section III.
2. Thaw Rapid Ligation Reaction Buffer (5×), Rapid T4 DNA Ligase, and Native Adapter on ice. Invert gently to mix, briefly centrifuge, and keep on ice. Prepare the reaction mixture in a PCR tube according to Table 5:

Table 5. Native Adapter Ligation PCR Reaction Setup

Components	Volume(μL)
Purified Native Barcode Ligation Product	30
Rapid Ligation Reaction Buffer (5×)	20
Native Adapter (NA)*	5
Rapid T4 DNA Ligase	5
ddH ₂ O	Up to 100

[Note]: Native Adapter (NA) is recommended to be an ONT official product (e.g., #EXP-NBA114).

- Gently pipette up and down to mix (do not vortex), then briefly centrifuge to collect the reaction mix at the bottom.
- Place the PCR tube in a thermal cycler and run the program as specified in Table 6:

Table 6. Native Adapter Ligation PCR Cycling Program

Temperature	Time
Hot lid to 105°C	Off
20°C	20 min
4°C	Hold

V. Purification of Native Adapter Ligation Products

This step purifies the final library using Hieff NGS™ DNA Selection Beads V2 (Cat# 12418).

- Preparation: Equilibrate magnetic beads at room temperature for 30 min. Retrieve ONT-compatible wash buffers (LFB or SFB).
 - Vortex or invert the bead suspension thoroughly before use.
 - Add 40 μL Hieff NGS™ DNA Selection Beads V2 (0.4× ratio) to the ligation reaction from Section IV. Pipette up and down 10 times to mix. Incubate at room temperature for 10 min.
 - Briefly centrifuge and place the tube on a magnetic rack. Once the supernatant is clear (~5 min), carefully remove and discard the supernatant.
 - Add 200 μL Long Fragment Buffer (LFB)* or Short Fragment Buffer (SFB)** to resuspend the beads. Pipette gently 10 times to mix. Return the tube to the magnet, wait for clarity, then remove the supernatant.
 - *Use LFB (Nanopore #EXP-LFB001)—recommended for enriching fragments ≥3 kb.
 - **Use SFB (Nanopore #EXP-SFB001)—recommended for retaining a broader size distribution.
- Do NOT use 80% ethanol in this step! (Buffer volume may be adjusted based on prior optimization.)
- Repeat Step 5 for a total of two washes.
 - While on the magnet, remove all residual supernatant.
 - Remove the tube from the magnet and add 18 μL EB buffer or elution buffer (10 mM Tris-HCl, pH 8.0–8.5) to cover the beads. Pipette to mix. Incubate at 37°C for 10 min. Extend incubation if beads are over-dried.
 - Place the tube back on the magnet and wait until the supernatant is clear (~5 min).
 - Transfer 16 μL of the supernatant to a new PCR tube. Use 1 μL for Qubit quantification. Store the final library at 4°C.

Troubleshooting Guide

1. Poor DNA quality (DIN < 5)

- ❖ Minimize mechanical shearing during extraction (e.g., reduce grinding or sonication time).
- ❖ Extend end-repair time or increase input DNA amount (do not exceed the maximum recommended input).

2. Low library yield

- ❖ Inaccurate bead volume or insufficient mixing during Native Barcode purification. Always vortex beads before use and avoid air bubbles or bead retention in tips.
- ❖ Over-drying of beads during purification. Proceed to elution once beads appear matte (not glossy).
- ❖ For long-fragment libraries, use wide-bore tips during mixing to prevent DNA shearing.

3. Low usable sequencing output

- ❖ Incomplete or insufficient Native Barcode/Adapter ligation. Consider extending ligation time or increasing reagent amounts.
- ❖ Incorrect wash buffer used in Native Adapter purification. Always use ONT-specified LFB or SFB, selected based on desired fragment size.
- ❖ Accidental transfer of magnetic beads during final elution. Ensure no beads are carried over into the eluate.



Note:

[illegible]

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