Micronbrane Medical

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RUO

Unison™ Ultralow DNA NGS Library Preparation Kit

REF NLK-01-048
(For Research Use Only)

1. DESCRIPTION

Unison™ Ultralow DNA NGS Library Preparation Kit is a set of next-generation sequencing library construction reagents suitable for ultralow input of DNA. It has the advantages of fast process, simple operation, ultralow input DNA samples required, consistent and good quality of final constructed library DNA samples, etc. Constructed DNA libraries can be sequenced on the Illumina Next-Generation Sequencing (NGS) platforms.

2. BEFORE USING THIS KIT

This NGS library kit is for Research Use Only (RUO).

- Please read the contents of this operation manual carefully before the experiment, including precautions, description of operation steps, etc. If you have any questions or concerns about this reagent kit, please contact us before starting the experiment.
- Please carefully confirm all the contents of this reagent kit are complete before the experiment. For the list of contents, please refer to 3. List of kit contents. If there is any component missing, please contact us as soon as possible.

3. LIST OF KIT CONTENTS

This NGS kit contains 1 box, including the following contents:

Box 1 (store at -20°C):

1. Transposase Mix x 1 tube (red cap)
2. 5x Reaction buffer x 1 tube (blue cap)
3. 5x Stop buffer x 1 tube (yellow cap)
4. Amplification Mix x 1 tube (green cap)

4. STORAGE

Box 1 reagents of this kit must be stored at -20°C. Follow the storage conditions and DON'T use after the expiration date indicated on the outer packaging.

5. SUPPLEMENTAL EQUIPMENT AND CONSUMABLES

The following items are required but not included in the kit. Please confirm the availability before starting the procedure.

- Disposable gloves
- Pipettes and compatible pipette tips
- Vortexer/Microcentrifuge
- Absolute Ethanol (200 proof) for molecular biology
- Nuclease-free water
- Index Primers (IDT® for Illumina® DNA/RNA UD Indexes or other compatible products)
- Disposable masks
- PCR Thermal Cycler
- 1.5ml microcentrifuge tubes
- 1.5ml Lobind Eppendorf tubes
- 0.5ml PCR tubes
- 0.5ml PCR tube magnetic stand
- Purification beads (Sera-Mag Select™ beads or other compatible products)

6. PRECAUTIONS

1. Please wear mask and gloves throughout the experiment.
2. Please use sterile and molecular biology grade nuclease free consumables to avoid sample degradation and contaminations.
3. Please wear long-sleeved clothes and trousers and avoid direct contact of reagents with the skin, eyes, mouth, and other parts. In case of any contact, flush with flowing water immediately.
4. Before the experiment, thaw the reagents at the following conditions:

   Thaw at **Room Temperature (RT)**:

   – Transposase Mix
   – 5x Reaction buffer
   – 5x Stop buffer
   – Purification beads (bring to RT at 30 minutes)

   Thaw on **Ice (Keep on ice all the time)**:

   – Amplification Mix
   – Index primers
5. Please do not expose the reagents in opened tubes for prolonged time to avoid contamination and degradation.

7. INPUT SAMPLE PREPARATION

This kit is suitable for ultralow input DNA, the recommended input DNA is **1 ng~5 ng** in a maximum volume of 8.6µl for each reaction. The recommended DNA quantification method is Qubit dsDNA high sensitivity kit or other fluorescence-based methods. Nanodrop and other absorbance-based DNA quantitation method is not recommended. If input DNA is not in the interval of recommended, please refer to 8.2.2 table to adjust amplification PCR cycle.

8. EXPERIMENTAL PROCEDURE

8.1 DNA fragmentation

8.1.1 Thaw Transposase Mix, 5x Reaction buffer, 5x Stop buffer (stored at -20°C) to RT. Bring Amplification Mix, index primers (stored at -20°C) on ice to thaw. Bring Purification beads to RT for at least 30 mins. Briefly vortex and spin all the reagents before use.

8.1.2 For each reaction, add 1µl Transposase Mix, 2.4 µl 5x Reaction buffer, DNA samples (1 ng-5 ng in a maximum volume of 8.6 µl) into a PCR tube. Top the reaction with nuclease-free water to 12 µl and mix well.

8.1.3 Place the reaction tube in PCR Thermal Cycler and run the fragmentation reaction at the following condition:

Lid Tm 105°C

Step	Tm (°C)	Time
1	55	5 mins
2	10	10 mins

8.1.4 Take out the reaction tube from PCR Thermal Cycler and add 3 µl 5x Stop Buffer into each reaction, mix well and put back to the cycler to stop the fragmentation at the following condition:

Lid Tm 105°C

Step	Tm (°C)	Time
1	55	5 mins

8.2 DNA amplification

8.2.1 Take out the reaction tube from PCR Thermal Cycler after finishing 8.1.4, add 15 µl Amplification Mix, 1 µl index primer, and mix well.

8.2.2 Place the reaction tube in PCR Thermal Cycler and run the amplification reaction at the following conditions:

Lid Tm 105°C

Step	Tm (°C)	Time	Cycle
1	72	3 mins	
2	98	30 secs	
3	98	30 secs	**14***
4	60	30 secs	
5	72	2 mins	
6	72	2 mins	
7	10	**∞**	

*** PCR cycle adjustment according to different DNA input**

DNA input	Cycle
1ng~5ng	14
5ng~50ng	12
≧ 50ng	10

1 step selection protocol for all of library size≧550bp
(if your sequencing platform is sensitive for large size library, then we suggest the following 2 step protocol for specific library size)

8.2.3 Take out the reaction tube from PCR Thermal Cycler, add 21 µl Purification beads, mix well gently and spin briefly, incubate at RT for 5 mins.

8.2.4 Place the tube on the magnetic stand for 5 mins or until the beads are fully separated, aspirate and discard the supernatant.

8.2.5 With the tube on the magnetic stand, wash the beads with 100 µl freshly prepared 80% Ethanol, do not disturb the beads pellet, and keep the tube on the magnetic stand for 30 sec before carefully remove the ethanol.

8.2.6 Repeat step 8.2.5

8.2.7 Place the tube on the magnetic stand with the lid open, dry the beads for ~ 7 mins at RT to remove any residual ethanol. Do not over-dry to beads till it cracks.

8.2.8 Remove the tube from the magnetic stand, add 11 µl nuclease-free water, mix well and place at RT for 2 mins.

8.2.9 Return the tube to the magnetic stand for 5 mins, take 10 µl supernatant to a new Lobind Eppendorf tube. The constructed library DNA can be stored at -20°C.

2 step selection protocol for specific library size

8.2.3 Please refer to the following table and choose a suitable library size. (The following condition is validated through Sera-Mag Select™ beads. If using other compatible products, please refer to the brand guide.)

Library average size	~350bp	~450bp	~550bp	~680bp
beads volume V1	0.7x	0.6x	0.55x	0.45x
beads volume V2	0.2x	0.15x	0.15x	0.15x

(The beads volume V1 & V2 are calculated by sample volume after **8.2.2 DNA amplification.** For example, the sample volume after **8.2.2 DNA amplification** is 31 µl and library target size is 550bp, then beads volume V1 should be 31 µl * 0.55 = 17 µl, and beads volume V2 should be 31 µl * 0.15 = 4.6 µl)

8.2.4 Take out the reaction tube from PCR Thermal Cycler, add beads volume V1 Purification beads, mix well gently and spin briefly, incubate at RT for 5 mins.

8.2.5 Place the tube on the magnetic stand for 5 mins or until the beads are fully separated, transfer the supernatant to new PCR tube.

8.2.6 Add beads volume V2 Purification beads into the supernatant, mix well gently and spin briefly, incubate at RT for 5 mins.

8.2.7 Place the tube on the magnetic stand for 5 mins or until the beads are fully separated, aspirate and discard the supernatant.

8.2.8 With the tube on the magnetic stand, wash the beads with 100 µl freshly prepared 80% Ethanol, do not disturb the beads pellet, and keep the tube on the magnetic stand for 30 sec before carefully remove the ethanol.

8.2.9 Repeat step 8.2.8

8.2.10 Place the tube on the magnetic stand with the lid open, dry the beads for ~ 7 mins at RT to remove any residual ethanol. Do not over-dry to beads till it cracks.

8.2.11 Remove the tube from the magnetic stand, add 11 µl nuclease-free water, mix well and place at RT for 2 mins.

8.2.12 Return the tube to the magnetic stand for 5 mins, take 10 µl supernatant to a new Lobind Eppendorf tube. The constructed library DNA can be stored at -20 °C.

9. WARNINGS ⚠

** The kit shall only be handled by educated personal in a laboratory environment!
** Handle reagents with special care and avoid any direct contact. In case of contact, flush eyes or skin immediately with a large amount of water.