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# Unison Ultralow DNA NGS Library Preparation Kit

NLK-01-008 NLK-01-048 For Research Use Only




## 1. Description

The Unison Ultralow DNA NGS Library Preparation Kit is part of Micronbrane Medical's Pathogen Real-Time Identification by Sequencing (PaRTI-Seq™) Metagenomic Next-Generation Sequencing (mNGS) assay. PaRTI-Seq delivers accurate, reliable and comprehensive pathogen identification from any liquid biopsy within 24 hours.

Unison enables library generation from ultra low DNA inputs (as low as 10 pg) while accelerating the workflow by combining fragmentation, adapter ligation, and normalization into a single step. The kit is specifically optimized for high molecular weight DNA and generates library fragments of optimal sizes and normalized concentrations for Illumina platforms.

## 2. Intended use

The Unison Ultralow DNA NGS Library Preparation Kit can be used for various applications including whole genome sequencing, targeted sequencing by capture, and mNGS. It is intended for research use only. It is not designed for medical, household, or general use and should only be handled by personnel with specialized knowledge in a laboratory environment.

## 3. Before starting

- Read this instruction manual carefully. If you have any questions, please contact support@micronbrane.com.
- Check that you have all the components. If something is missing, contact us immediately and do NOT proceed.

## 4. Contents

The Unison Kit includes one box with the followng contents:

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| | |
|---|---|
| 1. Transposase Mix | 1 tube **(red cap)** |
| 2. 5x Reaction Buffer | 1 tube **(blue cap)** |
| 3. 5x Stop Buffer | 1 tube **(purple cap)** |
| 4. 5x Amplification Mix | 1 tube **(clear cap)** |

## 5. Storage

- Store all other reagents at -20°C.
- DO NOT use after the expiration date as indicated on the outer packaging.

## 6. Supplemental Equipment and Consumables

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

- Index primers (IDT® for Illumina® DNA/RNA UD Indexes or other compatible products)
- Purification beads (Sera-Mag Select™ beads or other compatible products)
- Disposable gloves
- Disposable masks
- Pipettes and compatible pipette tips
- Vortex
- Microcentrifuge
- Absolute ethanol (molecular biology-grade)
- Nuclease-free water
- PCR thermal cycler
- 1.5 ml microcentrifuge tubes
- 1.5 ml DNA low binding tubes
- 0.6 ml microcentrifuge tubes
- Magnetic stand
- Qubit™ dsDNA High Sensitivity Assay Kit
- 0.2 ml PCR tube magnetic stand

## 7. Warnings and Precautions

⚠ Clinical samples may be potentially infectious.

⚠ Samples from at-risk patients must be labeled and handled in accordance with laboratory safety protocols.

⚠ Library preparation should only be performed by properly trained personnel.

⚠ When handling reagents, wear a mask, disposable gloves, and protective clothing.

⚠ Avoid contact with eyes, skin, and clothing. In case of contact, immediately flush the affected area with flowing water.

⚠ Use sterile, molecular biology-grade nuclease-free consumables to prevent sample degradation and contamination.

## 8. Input Sample Preparation

- Quantify the extracted DNA using Qubit dsDNA High Sensitivity Assay Kit or other fluorescence-based methods. Nanodrop and other absorbance-based DNA quantitation methods are not recommended.
- The recommended input DNA is 1 ~5 ng or as low as 10 pg in a maximum volume of 8.6µl for each reaction.
  *Note:* If you encounter difficulties with library construction using clinical samples, consider reducing the DNA input to 2 µl to mitigate potential PCR interference.
- If the final library is below the detection limit of Qubit, it may still be possible to sequence the library on the Illumina sequencing platform.

## 9. Fragmentation process

9.1 Thaw the reagents

- Thaw the Transposase Mix, 5x Reaction Buffer and 5x Stop Buffer (stored at -20°C) at room temperature.
- Thaw the purification beads at room temperature for at least 30 minutes.
- Thaw the 5x Amplification Mix and index primers on ice. *(Keep them on ice at all times.)*
- Briefly vortex and spin down all the reagents before use.

9.2 Set up the reaction mix on ice according to Table 1. Mix well by pipetting. Do not vortex.

**Table 1. Fragmentation reaction mix**

| *Reagent* | *Volume/µl* |
|---|---|
| Transposase Mix | 1 |
| 5x Reaction Buffer | 2.4 |
| DNA samples (1-5 ng) | x |
| Nuclease-free water | 8.6-x |
| *Total reaction volume* | *12* |

9.3 Place the reaction tube in the thermal cycler and run the fragmentation reaction according to Table 2.

**Table 2. Fragmentation reaction PCR conditions**

| *Step* | *Temperature/°C* | *Time/min* |
|---|---|---|
| 1 | 55 | 5 |
| 2 | 10 | 10 |

Lid temperature 105°C

9.4 When the reaction is complete, immediately remove the reaction tube from the thermal cycler and add 3 µl of 5x Stop Buffer. Mix well and put back the tube into the thermal cycler to stop the fragmentation reaction according to Table 3.

**Table 3. Stop reaction**

| *Step* | *Temperature/°C* | *Time/min* |
|---|---|---|
| 1 | 55 | 5 |

Lid temperature 105°C

## 10. DNA Amplification Reaction

10.1 Remove the reaction tube from the thermal cycler and prepare the DNA amplification reaction mixture according to Table 4. Pipette to mix.

**Table 4. DNA amplification reaction mix**

| *Reagent* | *Volume/µl* |
|---|---|
| Fragmented DNA | 15 |
| 5x Amplification Mix | 6 |
| Index primers | 1 |
| Nuclease-free water | 8 |
| *Total reaction volume* | *30* |

10.2 Place the reaction tube in the thermal cycler and run the amplification reaction according to Table 5.

**Table 5. DNA amplification reaction PCR conditions**

| *Step* | *Temperature/°C* | *Time/min* | *Cycle* |
|---|---|---|---|
| 1 | 72 | 3 | 1 |
| 2 | 98 | 0.5 | 1 |
| 3 | 98 | 0.5 | |
| 4 | 60 | 0.5 | 14* |
| 5 | 72 | 2 | |
| 6 | 72 | 2 | |
| 7 | 10 | ∞ | Hold |

Lid Temperature 105°C

*Cycle number can be adjusted according to DNA input. See Table 6.

**Table 6. Recommended number of PCR cycles**

| *DNA input* | *No of cycles* |
|---|---|
| <1 ng | 18 |
| 1-5 ng | 14 |
| 5-50 ng | 12 |
| >50 ng | 10 |

10.3 Please refer to Table 7 to calculate purification beads volume, V1 and V2. Beads volume calculations depend on the target library size and the final sample volume after the DNA amplification step in 10.2.

**Table 7. Beads volume calculation**

| *Average Library Size* | *~350bp* | *~450bp* | *~550bp* | *~680bp* |
|---|---|---|---|---|
| Beads volume 1 (V1) | 0.7x | 0.6x | 0.55x | 0.45x |
| Beads volume 2 (V2) | 0.2x | 0.15x | 0.15x | 0.15x |

*Example: If final sample volume = 30 µl and the target library size = 550 bp, then V1 = 30 µl x 0.55 = 16.5 µl and V2 = 30 µl x 0.15 = 4.5 µl.*

10.4 Add V1 of purification beads to the PCR tube containing the amplified DNA library. Mix well and spin down briefly. Incubate at room temperature for 5 minutes.

10.5 Place the tube on the magnetic stand for 5 minutes until all beads have been pelleted. Carefully transfer the supernatant to a new PCR tube.

10.6 Add V2 of purification beads to the supernatant. Mix well and spin down briefly. Incubate at room temperature for 5 minutes.

10.7 Place the tube on the magnetic stand for 5 minutes until all beads have been pelleted. Carefully aspirate and discard the supernatant.

10.8 While keeping the tube on the magnetic stand, wash the beads with 100 µl freshly prepared 80% ethanol without disturbing the beads pellet. Keep the tube on the magnetic stand for 30 seconds before carefully aspirating and discarding the ethanol with a pipette.

10.9 Repeat step 10.8.

10.10 Place the tube on the magnetic stand with the lid open. Allow the beads to air dry at room temperature for ~ 7 minutes, or until visible cracking occurs, whichever comes first, to ensure complete evaporation of any residual ethanol.

10.11 Remove the tube from the magnetic stand and add 11 µl nuclease-free water to elute DNA from the beads. Mix well and incubate at room temperature for 2 minutes.

10.12 Return the tube to the magnetic stand for 5 minutes. Carefully transfer 10 µl of the supernatant to a new low-binding tube. The constructed DNA library can be stored at -20 °C.

## 11. Specifications

- **Packaging:** NLK-01-008/048: 4 tubes per box
- **Shelf Life:** 1 year at -20°C

## 12. Disposal Instructions

Dispose in accordance with local rules and regulations for hazardous materials.