GDSNext Non-Directional RNA Second Strand Synthesis Module

Instruction for Use

【Product Name】

GDSNext Non-Directional RNA Second Strand Synthesis Module

【Cat. No./Spec.】

K022-A/20 rxns; K022-B/100 rxns

【Product Description】

The GDSNext Non-Directional RNA Second Strand Synthesis Module, an optimized non-specific RNA second-strand synthesis module, can utilize the first-strand cDNA synthesized by the GDSNext RNA First Strand Synthesis Module (# K020) to generate double-stranded cDNA. The dsDNA produced by the GDSNext Non-Directional RNA Second Strand Synthesis Module can then be converted into blunt-end DNA fragments using the GDSNext End Repair/dA-Tailing Module (# K025).

【Components】

Component	*K022-A*	*K022-B*
GDSNext Second Strand Synthesis Enzyme Mix	*80 μL*	*400 μL*
GDSNext Second Strand Synthesis Reaction Buffer	*160 μL*	*800 μL*

【Storage Condition & Shelf Life】

All reagents should be stored at -20°C. The product is valid for 24 months.

【Application】

For the synthesis of the second-strand cDNA of RNA in NGS library preparation.

【Protocol】

Starting Materials: 20 μL of first-strand cDNA synthesized using the GDSNext RNA First Strand Synthesis Module

1. Synthesis of second-strand cDNA

1.1 Add the following components to the first-strand synthesis reaction product and assemble the second-strand cDNA synthesis reaction on ice:

Component	*Volume*
First-strand synthesis product	*20 μL*
GDSNext Second Strand Synthesis Reaction Buffer	*8 μL*
GDSNext Second Strand Synthesis Enzyme Mix	*4 μL*
Nuclease-free Water	*48 μL*
Total volume	*80 μL*

1.2 Keep the PCR tube on ice and gently pipette up and down at least 10 times with a pipette to mix the entire volume uniformly.

1.3 Place the sample in a thermal cycler and incubate at 16° C for 1 hour, with the hot lid set to ≤ 40° C (or turned off).

2. Purify double-stranded cDNA using GDSPure DNA Selection Magbeads

2.1 Vortex the GDSPure DNA Selection Magbeads to thoroughly mix the magnetic beads.

2.2 Add 144 μL (1.8X) of the magnetic bead suspension to the second-strand synthesis product (~80 μL), gently pipette 10 times (or vortex for 30s), and let it stand at room temperature for 5 minutes.

2.3 Place the centrifuge tube on a magnetic rack until the solution becomes clear. Use a pipette to aspirate and discard the supernatant, taking care not to aspirate the magnetic beads.

2.4 While keeping the centrifuge tube on the magnetic rack, add 200 μL of 80% ethanol without disturbing the magnetic beads. After letting it stand at room temperature for 30s, use a pipette to aspirate and discard the supernatant.

2.5. Repeat step 2.4 once.

Note: After the final wash, ensure to aspirate the wash buffer as cleanly as possible.

2.6. Keep the centrifuge tube on the magnetic rack and allow the magnetic beads to air-dry for 5 minutes until the surface of the beads loses its noticeable shine.

Note: This step should avoid over-drying the magnetic beads, which can affect the elution efficiency. Cracks on the surface of the beads indicate over-drying.

2.7. Remove the centrifuge tube from the magnetic rack, add 53 μL of 0.1X TE buffer to the tube, and pipette gently at least 10 times (or vortex for 30s) to thoroughly mix the magnetic beads with the solution. Let it stand at room temperature for 2 minutes.

2.8. Place the centrifuge tube on the magnetic rack until the solution becomes clear. Transfer 50 μL of the supernatant to a new centrifuge tube, and the purification is complete.

2.9. The product can be stored at -20°C or used for end repair with the GDSNext End Repair/dA-Tailing Module (# K025).

【Library Preparation Module】

GDSBio offers the following DNA and RNA library construction modules that can be used in combination for high-quality library preparation:

Module	Product Name	Cat. No./Spec.
cDNA First Strand Synthesis	*GDS RNA First Strand Synthesis Module*	*K020-A/24 rxns* *K020-B/96 rxns*
Directional cDNA Second Strand Synthesis	*GDS Directional RNA Second Strand Synthesis Module*	*K021-A/24 rxns* *K021-B/96 rxns*
Non-Directional cDNA Second Strand Synthesis	*GDS Non-Directional RNA Second Strand Synthesis Module*	*K022-A/20 rxns* *K022-B/100 rxns*
Fragmentation & End Repair	*GDS Fragmentation & End Prep Module*	*K023-A/24 rxns* *K023-B/96 rxns*
Fragmentation	*GDS dsDNA Fragmentase*	*K024-A/50 rxns* *K024-B/250 rxns*
End Repair/dA-Tailing	*GDS End Preparation Module*	*K025-A/24 rxns* *K025-B/96 rxns*
Adapter Ligation	*GDS Ligation Module*	*K026-A/24 rxns* *K026-B/96 rxns*
Amplification	*HIFI Library PCR Master Mix*	*K007-A/40 rxns* *K007-B/400 rxns* *K007-C/2000 rxns*
Cleanup/Size Selection	*GDSPure DNA Selection Magbeads*	*NC1011/5 mL* *NC1012/60 mL* *NC1013/450 mL*

This product is for research use only.