

GDS Directional RNA Second Strand Synthesis Module Instruction for Use

【Product Name】

GDS Directional RNA Second Strand Synthesis Module

【Cat. No./Spec.】

K021-A/24 rxns; K021-B/96 rxns

【Product Description】

The GDSNext Directional RNA Second Strand Synthesis Module, a specific RNA second-strand synthesis module, has been optimized to generate double-stranded cDNA using the first-strand cDNA synthesized by the GDSNext RNA First Strand Synthesis Module (# K020). The dsDNA produced by the GDSNext 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	K021-A	K021-B
GDSNext Second Strand Synthesis Enzyme Mix	96 μ L	384 μ L
GDSNext Second Strand Synthesis Reaction Buffer with dUTP Mix	192 μ L	768 μ L

【Storage Condition & Shelf Life】

All reagents should be stored at -20°C . The product is valid for 18 months.

【Application】

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

【Protocol】

Starting Materials: 20 μ L 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 μ L
GDSNext Second Strand Synthesis Reaction Buffer with dUTP Mix	8 μ L

GDSNext Second Strand Synthesis Enzyme Mix	4 μ L
Nuclease-free Water	48 μ L
Total volume	80 μ L

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 $\leq 40^{\circ}\text{C}$ (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 μ L (1.8X) of the magnetic bead suspension to the second-strand synthesis product (~80 μ L), 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 μ L 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 μ L 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 μ L 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.
<i>cDNA First Strand Synthesis</i>	<i>GDS RNA First Strand Synthesis Module</i>	<i>K020-A/24 rxns K020-B/96 rxns</i>
<i>Directional cDNA Second Strand Synthesis</i>	<i>GDS Directional RNA Second Strand Synthesis Module</i>	<i>K021-A/24 rxns K021-B/96 rxns</i>
<i>Non-Directional cDNA Second Strand Synthesis</i>	<i>GDS Non-Directional RNA Second Strand Synthesis Module</i>	<i>K022-A/20 rxns K022-B/100 rxns</i>
<i>Fragmentation & End Repair</i>	<i>GDS Fragmentation & End Prep Module</i>	<i>K023-A/24 rxns K023-B/96 rxns</i>
<i>Fragmentation</i>	<i>GDS dsDNA Fragmentase</i>	<i>K024-A/50 rxns K024-B/250 rxns</i>
<i>End Repair/dA-Tailing</i>	<i>GDS End Preparation Module</i>	<i>K025-A/24 rxns K025-B/96 rxns</i>
<i>Adapter Ligation</i>	<i>GDS Ligation Module</i>	<i>K026-A/24 rxns K026-B/96 rxns</i>
<i>Amplification</i>	<i>HIFI Library PCR Master Mix</i>	<i>K007-A/40 rxns K007-B/400 rxns K007-C/2000 rxns</i>
<i>Cleanup/Size Selection</i>	<i>GDSPure DNA Selection Magbeads</i>	<i>NC1011/5 mL NC1012/60 mL NC1013/450 mL</i>

This product is for research use only.