GDSNext RNA First Strand Synthesis Module

Instruction for Use

【Product Name】

GDSNext RNA First Strand Synthesis Module

【Cat. No./Spec.】

K020-A/24 rxns; K020-B/96 rxns

【Product Description】

The GDSNext RNA First Strand Transcription Module is an optimized RNA first-strand transcription module that can transcribe a wide range of input RNA into cDNA using random primers.

This module can be used in conjunction with the GDSNext Directional RNA Second Strand Synthesis Module (# K021) for specific RNA second-strand synthesis or the GDSNext Non-Directional RNA Second Strand Synthesis Module (# K022) for non-specific RNA second-strand synthesis. These workflows are compatible with poly(A) mRNA isolation or ribosomal RNA depletion and enable high-yield preparation of high-quality libraries from 10 ng to 1 μg of total RNA.

【Components】

Component	*K020-A (24 rxns)*	*K020-B (96 rxns)*
GDSNext First Strand Synthesis Enzyme Mix	*192 μL*	*768 μL*
GDSNext First Strand Synthesis Reaction Buffer	*48 μL*	*192 μL*
Random Primers	*48 μL*	*192 μL*
GDSNext Strand Specificity Reagent	*192 μL*	*768 μL*

【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 first-strand cDNA of RNA in NGS library preparation.

【RNA Sample Recommendations】

RNA Integrity:

The RNA Integrity Number (RIN) is calculated using the quantity of ribosomal RNA (rRNA) in the sample. If rRNA is removed by any method, the RIN value should not be used to assess the integrity of the RNA sample. In such cases, we recommend that if the RNA sample is suspected to be of low quality, the fragmentation time needs to be validated. The following recommendations apply only to total RNA samples.

The quality of input RNA is assessed by running the RNA sample on an Agilent Bioanalyzer RNA 6000 Nano/Pico Chip to determine the RNA RIN. RNAs with different RIN values require different fragmentation times or may not require fragmentation at all. For highly degraded samples (RIN = 1 to 2) such as FFPE, no fragmentation is necessary.

RNA Purity:

RNA samples should be free of DNA, salts (such as Mg2+ or guanidinium salts), divalent cation chelators (such as EDTA, EGTA, citrate), or organics (such as phenol and ethanol).

Input Quantity Requirements:

1 ng - 100 ng of total RNA, purified mRNA, or rRNA-depleted RNA after purification and quantification. The RNA should be free of DNA, quantified using a Qubit Fluorometer in 5μL of nuclease-free water, and quality-checked with a Bioanalyzer.

This protocol is optimized for approximately 200 nt RNA inserts. To generate libraries with longer RNA insert lengths, please refer to other recommended fragmentation times.

【Protocol】

Note: This protocol is applicable only to total RNA, purified mRNA, or rRNA-depleted RNA.
1.1. RNA Fragmentation and Primer Binding
Intact or partially degraded RNA requires fragmentation. The recommended fragmentation times are provided in Table 1.
1.1.1. In a nuclease-free tube, prepare the fragmentation and primer binding reaction mixture on ice by adding the following components:

Component	*Volume*
Purified mRNA, or rRNA-depleted RNA	*5 μL*
GDSNext First Strand Synthesis Reaction	*4 μL*

Buffer	
Random Primers	*1 μL*
Total volume	*10 μL*

1.1.2 Pipette gently up and down at least 10 times to thoroughly mix the entire volume.

1.1.3. Place the sample in a thermocycler and incubate it at 94°C for the recommended fragmentation time based on the RIN value of the RNA, as shown in Table 1, to achieve an insert size of approximately 200 nt.

Table 1: Recommended Fragmentation Times Based on RNA RIN Value

RNA type	*RIN value*
Intact RNA	*> 7*
Partially degraded RNA	*2~6*

1.1.4. Immediately transfer the PCR tubes to ice and proceed with the first-strand cDNA synthesis.

1.2 First-strand cDNA Synthesis Reaction

Note: Depending on the subsequent second-strand cDNA synthesis module, choose either Protocol A or Protocol B.

Protocol A: Procedure for use with the GDSNext Directional RNA Second Strand Synthesis Module (# K021)

1.2.1. Assemble the first-strand synthesis reaction on ice by adding the following components to the fragmented and primer-bound RNA from step 1.1.4:

Component	*Volume*
Fragmented and primer-bound RNA（1.1.4）	*10 μL*
GDSNext Strand Specificity Reagent	*8 μL*
GDSNext First Strand Synthesis Enzyme Mix	*2 μL*
Total volume	*20 μL*

1.2.2 Pipette gently up and down at least 10 times to thoroughly mix the entire volume.

1.2.3 Set the hot lid of the PCR machine to ≥80° C, preheat, and then perform the following reaction:

Temperature	*Time*
25℃	*10 min*
42℃	*15 min*
70℃	*15 min*
4℃	*hold*

Note: If you are using longer RNA fragments (>200 nt), please increase the incubation time at 42° C in the second step from 15 minutes to 50 minutes.

1.2.4. Proceed directly to second-strand cDNA synthesis using the GDSNext Directional RNA Second Strand Synthesis Module (# K021).

Protocol B: Procedure for use with the GDSNext Non-Directional RNA Second Strand Synthesis Module (# K022)

1.2.1. Assemble the first-strand synthesis reaction on ice by adding the following components to the fragmented and primer-bound RNA from step 1.1.4:

Component	*Volume*
Fragmented and primer-bound RNA（1.1.4）	*10 μL*
Nuclease-free Water	*8 μL*
GDSNext First Strand Synthesis Enzyme Mix	*2 μL*
Total volume	*20 μL*

1.2.2 Pipette gently up and down at least 10 times to thoroughly mix the entire volume.

1.2.3 Set the hot lid of the PCR machine to ≥80° C, preheat, and then perform the following reaction:

Temperature	*Time*
25℃	*10 min*
42℃	*15 min*
70℃	*15 min*
4℃	*hold*

Note: If you are using longer RNA fragments (>200 nt), please increase the incubation time at 42° C in the second step from 15 minutes to 50 minutes.

1.2.4. Proceed directly to second-strand cDNA synthesis using the GDSNext Non-Directional RNA Second Strand Synthesis Module (# K022).

【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	*GDS Non-Directional RNA Second Strand*	*K022-A/20 rxns*

Strand Synthesis	*Synthesis Module*	*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.