First Strand cDNA Synthesis Kit II

(Cat. No.: S2GNM03j30002)

Description:

First Strand cDNA Synthesis Kit II contains recombinant MMLV reverse transcriptase with improved thermostability and reduced RNase H activity. It is an easy-to-use kit for reliable cDNA synthesis, which is able to synthesize the first strand cDNA at 37~50°C.

This kit includes RNase Inhibitor, which effectively inhibits RNase A, RNase B, and RNase C activities, ensuring the integrity of RNA during the reverse transcription process. The product comes with an optimized RT Buffer and an Oligo (dT)/Random Primer Mix, enabling the highly efficient synthesis of short-chain cDNA that is suitable for real-time PCR applications.

Kit Contents:

Contents	S2GNM03j30002 (100 rxns)
RTase/RI Enzyme Mix	100 µl x 1
5X RT Buffer (DTT/dNTPs)	500 µl x 1
Oligo (dT)/Random Primer Mix	100 µl x 1
DEPC-Treated H_2O	1 ml x 2

Storage:

The kit is stable for 24 months at -20°C.

Protocol:

1. After thawing, mix and briefly centrifuge the components of the kit, keep it on ice.
2. Add the following reagents into a PCR tube and keep it on ice.

Table 1. Reaction Setup - Denature (Mixture A)	
Components	Volume
RNA template	X µl (1ng~2 µg)
Oligo (dT)/Random Primer Mix	1 µl
DEPC-Treated H_2O	to a final volume of 10 µl

Mix well and heated at 70°C for 5 minutes in advance, then incubated on ice bath at least 1 minute. Then, add other components according to the table.

Table 2. Reaction Setup - First strand cDNA buffer (Mixture B) per reaction (This Master Mix can be prepared before or during the denaturing step)	
Components	Volume
5X RT Buffer (DTT/dNTPs)	4 µl
DEPC-Treated H_2O	5 µl
RTase/RI Enzyme Mix	1 µl
Final volume	**10 µl**

3. Mix the following reaction mixture, then incubate at 25°C for 10 minutes and 37-50°C for 50 minutes.

Table 3. Reaction Setup	
Components	Volume
Mixture A	10 µl
Mixture B	10 µl
Final volume	**20 µl**

4. Incubate at 85°C for 5 minutes for Termination.
5. Store cDNA at -20°C or for immediate real-time PCR reaction

Recommended real-time PCR Condition

Table 4. Reaction Setup for real-time PCR	
Components	Volume
cDNA	2 µl (100 fg -100 ng) *

Forward primer	50 – 400 nM**
Reverse primer	50 – 400 nM**
2X qPCR Master Mix	10 µl
H_2O	to a final volume of 20 µl
Final volume	**20 µl**

* The volume of cDNA should not be more than 10% of the total qPCR reaction volume.

** The PCR primer concentration for an optimal qPCR reaction may vary based on the properties and the template of the primers.

Recommended real-time PCR Program

Table 5. Two-step Cycle			
Step	Temperature	Time	Cycles
Template denaturation and enzyme activation	95°C	10 mins#	1
Denaturation	95°C	15 sec	40
Annealing and Extension	60°C	60 sec	
Melting curve analysis	Refer to the instrument manual		

10 minutes is recommended for the first step to completely denature the DNA and activate the enzyme.

Recommended PCR Condition

Table 4. Reaction Setup	
Components	Volume
cDNA	2-10 µl
10X *Taq* Buffer	5 µl
Forward primer	0.1 – 0.5 µM
Reverse primer	0.1 – 0.5 µM
dNTPs	0.2 mM each
Taq DNA polymerase	0.25 µl (1.25 units)
H_2O	to a final volume of 50 µl
Final volume	**50 µl**

Recommended PCR Program

Table 5. Thermal Cycling Program			
Step	Temperature	Time	
Initial denaturation	94°C	2 mins	
Denaturation	94°C	30 sec	25-40 cycles
Annealing	50-68°C ##	30 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	1 min	

The optimal PCR conditions differ based on the thermodynamic properties of the primers.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	S2GNM03j30002_Protocol_V1	Aug 2023