First stranded cDNA Synthesis Kit (SAMscript)

Cat. No.: GRS01-M
Size: 20 reactions
Store at: -20°C

For research use only

Description:

The GeneMark **First stranded cDNA Synthesis Kit (SAMscript)** is provided with all components required to perform first-strand cDNA synthesis. SAMscript Reverse Transcriptase (SAMscript RTase) is genetically engineered version of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RTase) which is a frequent choice for cDNA synthesis because of its ease of use and low intrinsic RNase H activity. In the cDNA synthesis step, RNA was reverse transcribed by SAMscript RTase to produce its cDNA up to 5 kb. The RNase Inhibitor supplied with the kit can protect RNA from degradation.

Components:

This kit is suitable to perform **20** reactions of first-strand cDNA synthesis.

Contents	Volume	Cap
1. SAMscript RTase (4000 U), 200U/μl	20 μl	**purple**
2. 10X SAMscript RT Buffer	200 μl	**purple**
3. 2 mM dNTP mixture	200 μl	**white**
4. 25 μM Oligo (dT)18 Primer	50 μl	**green**
5. 50 μM Random Primer	100 μl	**yellow**
6. RNase Inhibitor (40 units/μl)	10 μl	**clear**
7. Nuclease-free H_2O	1.8 ml	**clear**

General Procedure:

1) **Sample denaturation:** Prepare the reaction mix by combining the indicated volumes as followed, then heat RNA/primer mixture at 70℃ for 5 min, and then cool immediately on ice for at least 3 min and store on ice.

Nuclease-free H_2O		X μl
RNA template (0.01 pg-5 μg)		Y μl
Primer *		1 μl
	Total	14.5 μl

* Template RNA: 10 ng-5 μg Total RNA, 1 ng-0.5 μg mRNA, 0.01 pg-0.5 μg Specific RNA

**Suggestion: each 10 μM gene specific primer; 25 μM Oligo dT Primer; 50 μM Random Primer

2) **cDNA synthesis:** Prepare the following reaction mix.

RNA/Primer mix on ice		14.5 μl
10X SAMscript RT Buffer		2 μl
SAMscript RTase		1 μl
2 mM dNTP Mix		2 μl
RNase Inhibitor (40 units/μl)		0.5 μl
	Total	20 μl

3) **Incubation condition of the cDNA synthesis reaction:** Based on the primer used in the reaction, use the incubation temperatures and times as below.

Oligo dT: 37~42℃ for 30~60 min.

Gene-Specific primer: 37~42℃ for 30~60 min.

Random Hexamers: 25℃ for 10 min, followed by 37~42℃ for 30~60 min.

4) **Terminate the Reaction:** Terminate the reaction by incubating at 72°C for 10 min.

***** The cDNA can be directly used in subsequent applications, or stored at -20°C for future use.

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