SinaClon First Strand cDNA Synthesis Kit

REF RT5201 — 50 TESTS

RUO — -20 — Wet or Dry ice

Components

Contents	Amounts
RT Enzyme Mix	50µl
5X Buffer M-MuLV	200µl
10mM dNTPs mix	50µl
Oligo(dT)$_{18}$	50µl
Random hexamer	50µl
DEPC-treated water	1ml

RT Enzyme Mix Components

Contents	Amounts
M-MuLV Reverse Transcriptase (200U/µl)	10000U
RNase Inhibitor (20U/µl)	1000U

Description

SinaClon First Strand cDNA synthesis Kit is specially designed to provide reliable synthesis of full-length cDNA from mRNA or total RNA templates. M-MuLV RNase-H synthesizes complementary DNA strand initiating from a specific primer, oligo(dT)$_{18}$ or random hexamer. The absence of RNase-H enhances the synthesis of long cDNA as the RNA strand does not degrade in DNA-RNA hybrid during first strand cDNA synthesis. The RNase inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55°C.

Storage and Stability

Stable at -20°C for 1 year if properly stored.

M-MuLV, Unit Definition

One unit of the enzyme incorporates 1nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA). Oligo(dT)$_{18}$.

RNase inhibitor, Unit Definition

One unit of the enzyme is defined as the amount of Ribonuclease inhibitor that inhibits the activity of 5ng Ribonuclease A by 50%.

Preliminary Considerations

Primers

Gene-specific primers, oligo(dT)$_{18}$ and random hexamer can be used in reverse transcription. Gene-specific primers transcribe only specific gene sequences and therefore increases the specificity. Gene-specific primers are used to synthesize specific cDNA from a pool of total RNA or mRNA and must be obtained by the user. Oligo(dT)$_{18}$ transcribes all poly (A) + mRNA, includes eukaryotic mRNA and viruses with poly(A) tail. Random hexamer initiate cDNA synthesis from the total RNA population (rRNA and mRNA). Therefore, using random primers for first strand synthesis results in a greater complexity of the generated cDNA compared with the oligo(dT)$_{18}$ primer. As a consequence, the sensitivity and specificity of subsequent PCR reactions may be reduced. However, there are several applications where it is beneficial to use random primers, such as cDNA synthesis using mRNAs without a poly(A) tail, or cDNA synthesis using poly(A)-enriched RNA samples.

RNA templates

Quality and quantity of RNA templates determine the efficiency of reverse transcription process. The presence of minute number of RNases can degrade the RNA and affect the cDNA length transcribed. To prevent RNases contamination, RNA purification has to be carried out in an RNase-free environment. Glassware, plasticware and reagents should be essentially RNase-free.

Protocol

Recommended protocol for first strand cDNA synthesis

1. After thawing, mix and briefly centrifuge the components of the kit, Store on ice.
2. Prepare the RNA-primer mixture as below in a sterile, nuclease-free tube on ice.

component	Volume/ Concentration
Template: total RNA	50ng-5µg
Primer: oligo(dT)$_{18}$ or random Hexamers or gene-specific primer	1µl 1µl Xµl (depends on the primer stock concentration (2pmol))
dNTP Mix (10Mm)	1µl
DEPC-treated water	Top up to 10µl

3. Incubate the mixture at 70°C for 5 minutes and chill on ice for 2 minutes.
4. Briefly spin down the mixture.
5. Prepare the following cDNA Synthesis Mix in the order indicated:

Component	Volume
5X Buffer M-MuLV	4µl
RT Enzyme Mix	1.2µl (200U RT +20U RNase Inhibitor)
DEPC-treated water	up to 10µl

6. Add 10µl of the cDNA Synthesis Mix into each RNA-primer mixture. Mix gently and centrifuge briefly.
7. Incubate at 37-50°C for 50 min.

Note: If random hexamer is used, incubation at 25°C for 10 min is needed prior to incubation at 37-50°C to prevent to dissociation of primer from template while the temperature increases. If gene specific primer or oligo(dT)$_{18}$ is used, this step can be ignored.

8. Terminate the reaction by incubate the tubes at 80°C for 5min. Chill the tubes on ice and collect the solution by centrifuge the tube briefly.
9. The synthesized cDNA can be directly used in PCR, by addition of 2-5µl of the cDNA reaction mixture to a 50µl PCR reaction.

Quality Control

RT-PCR using 200ng of control GAPDH RNA and GAPDH control primers generated a visible product on agarose gel after DNA Safe Stain staining.

Signs

Signs	Definitions	Signs	Definitions
-20	Temperature range on product use	REF	Product technical code
RUO	For Research Use Only		Product shipping conditions
	Name and address of the manufacturer of the product		

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