CinnaGen First Strand cDNA synthesis Kit

Cat. No.: RT5201 **Quantity: 50 reaction**
Store at: -20°C **Shipment: Wet/ Dry ice**

Description

CinnaGen 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 d(T) 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.

Kit Components(50 reaction):

M-MuLV Reverse Transcriptase(200u/µl)	**5000u**
RNase inhibitor(40u/µl)	**1000u**
10X Buffer M-MuLV	**100 µl**
10mM dNTPs mix	**100 µl**
Oligo $d(T)_{18}$ (100µM, 0.5µg/µl)	**50µl**
Random hexamer (100µM, 0.2µg/µl)	**50µl**
DEPC-treated water	**1ml**

Storage and Stability

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

M-MuLV Storage Buffer:
200mM NaCl, 10mM potassium phosphate (pH7.5), 0.1mM EDTA, 7mM 2-mercaptoethanol and 50% glycerol.

10X Buffer M-MuLV:
500mM Tris-HCl (pH8.3 at 25°C), 750mM KCl, 30mM MgCl2 and 100mM DTT.

RNase inhibitor Storage Buffer:
20mM HEPES-KOH (pH 7.6), 50mM KCl, 5mM DTT, and 50% glycerol.

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

RNase inhibitor, Unit Definition:
1u 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 d(T) and random hexamer can be used in reverse transcription. Gene-specific primer 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 d(T) transcribes all poly(A)+ mR NA, 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 amount of RNases can degrade the RNA and affect the cDNA length transcribed. To prevent RNases contamination, RNA purification have to be carried out in a RNase-free environment. Glassware, plasticware and reagents should be essentially RNase-free.

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 or poly A(+) mRNA	1 – 5µg 0.01 – 2µg
Primer: oligo $d(T)_{18}$ or random Hexamers or gene-specific primer	1µl 1µl Volume depends on the primer stock concentration (15-20 pmol)
DEPC-treated water	Top up to10µl

3. Incubate the mixture at 65°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
10X Buffer M-MuLV	2µl
M-MuLV Reverse Transcriptase	0.5µl
RNase inhibitor	0.5µl(20 u)
10mM dNTP Mix	2µl
DEPC-treated water	Top up to 10µl

6. Add 10µl of the cDNA Synthesis Mix into each RNA-primer mixture. Mix gently and centrifuge briefly.
7. Incubate at 42°C for 60 min.
Note:If randon hexamer is used, incubation at 25°C for 10 min is needed prior to incubation at 42°C to prevent to dissociation of primer from template while the temperature increases. If gene specific primer or oligo d(T) is used, this step can be ignored.

8. Terminate the reaction by incubate the tubes at 85°C for 5 min. 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 2-5 µl of the cDNA reaction mixture to a 25 µl PCR reaction.

Quality Control
RT-PCR using 100ng of control GAPDH RNA and GAPDH control primers generated a 496 bp product visible on agarose gel after ethidium bromide staining.