SinaClon First Strand
cDNA Synthesis Kit
For Research Use Only

Cat. No.: RT٥٢٠١ **Quantity: ٥٠ Reactions**
Store at: -٢٠°C **Shipment: Wet or Dry ice**

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)١٨ 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 ٥٥°C.

Kit Components(٥٠ reactions):

M-MuLV Reverse Transcriptase (٢٠٠u/µl)	**٥٠٠٠u**	
RNase Inhibitor (٤٠u/µl)	**١٠٠٠u**	
٥X Buffer M-MuLV		**٢٠٠ µl**
DTT (٠,١M)	**٥٠ µl**	
١٠mM dNTPs mix		**١٠٠ µl**
Oligod(T)١٨	**٥٠ µl**	
Random hexamer	**٥٠ µl**	
DEPC-treated water	**١ ml**	

Storage and Stability
Stable at -٢٠°C for ١ year if properly stored.

M-MuLV Storage Buffer:
٢٠ mM Tris-HCl (pH ٧,٥), ٢٠٠mM NaCl, ٠,١mM EDTA, ١mM DTT, Stabilizer, ٥٠٪ (v/v) glycerol.

٥X Buffer M-MuLV:
٢٥٠ mM Tris-HCl (pH٨,٣ at ٢٥°C), ٣٧٥ mM KCl, ١٥mM MgCl٢.

RNase inhibitor Storage Buffer:
٢٠mM HEPES-KOH (pH ٧,٦), ٥٠mM KCl, ٥mM DTT, and ٥٠٪ glycerol.

M-MuLV, Unit Definition:
One unit of the enzyme incorporates ١nmol of dTTP into an acid-insoluble material in ١٠ minutes at ٣٧°C using poly(rA). Oligo(dT)١٨.

RNase inhibitor, Unit Definition:
١u is defined as the amount of Ribonuclease inhibitor that inhibits the activity of ٥ng Ribonuclease A by ٥٠٪.

Preliminary Considerations:
Primers
Gene-specific primers, oligo(dT)١٨ 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) ١٨ 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)١٨ 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 has to be carried out in an RNase-free environment. Glassware, plasticware and reagents should be essentially RNase-free.

Recommended protocol for first strand cDNA synthesis

١. After thawing, mix and briefly centrifuge the components of the kit, Store on ice.
٢. 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	١ – ٥μg ٠,٠١ – ٢μg
Primer: oligo(dT)١٨ or random Hexamers or gene-specific primer	١μl ١μl Volume depends on the primer stock concentration(١٥-٢٠ pmol)
DEPC-treated water	Top up to١٠μl

٣. Incubate the mixture at ٦٥°C for ٥ minutes and chill on ice for ٢ minutes.
٤. Briefly spin down the mixture.
٥. Prepare the following cDNA Synthesis Mix in the order indicated:

Component	Volume
٥X Buffer M-MuLV	٤μl
DTT	١μl
M-MuLV Reverse Transcriptase	٠,٥μl
RNase inhibitor	٠,٥μl(٢٠u)
١٠mM dNTP Mix	٢μl
DEPC-treated water	Top up to ١٠μl

٦. Add ١٠μl of the cDNA Synthesis Mix into each RNA-primer mixture. Mix gently and centrifuge briefly.
٧. Incubate at ٤٢°C for ٦٠ min.

Note: If random 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 (dT)$_{18}$ is used, this step can be ignored.

٨. Terminate the reaction by incubate the tubes at ٨٥°C for ٥ min. Chill the tubes on ice and collect the solution by centrifuge the tube briefly.

٩. The synthesized cDNA can be directly used in PCR, by addition of ٢-٥μl of the cDNA reaction mixture to a ٢٥μl PCR reaction.

Quality Control

RT-PCR using ١٠٠ng of control GAPDH RNA and GAPDH control primers generated a ٤٩٦bp product visible on agarose gel after DNA Safe Stain staining.

SinaClon BioScience
www.sinaclon.com
Tel: +٩٨(٠)٢١ ٤٤٦٣ ٠٠٥٠-٥١
+٩٨(٠)٢١ ٤٤٦٦ ٥١٥٦
Mob.:+٩٨٩٠٢٣١٢٠٠٥٢,٥٤,٥٦
Customer Service: +٩٨(٠)٩٠٢٣١٢٠٠٥٩