



SuperiorScript III cDNA Synthesis Kit

Instruction Manual

Cat.# EZ405S, EZ405M

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SuperiorScript III cDNA Synthesis Kit

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I. Description

SuperiorScript III Reverse Transcriptase is genetically engineered version of M-MLV RT with reduced RNase H activity and increased thermostability, thus this enzyme can synthesize first-strand cDNA at elevated temperatures up to 55 °C. SuperiorScript III Reverse Transcriptase shows improved thermostability and reduced RNase H activity than the competitor's enzyme. This enzyme is capable of synthesizing first strand cDNA at 42°C~55°C with high specificity and yield. Synthesis of longer products (12 kb or more) are possible than the previous versions.

cDNA synthesis can be performed using oligo dT, random primer or gene specific primer with total RNA or mRNA. PCR reaction can be performed using specific primer for the target gene. One of the following PCR polymerase can be used for PCR reaction:

- *n*Taq-HOT (Cat.# P725) can be used to amplify specific target DNA up to 3 kb in hot start condition.
- *n*Taq-Tenuto (Cat.# P225) can be used to amplify longer DNA up to 15 kb with high yield and fidelity.
- *n*Pfu-Forte (Cat.# P410) can be used to amplify high fidelity DNA for cloning.
- *n*Pfu-Special (Cat.# P100) can be used to amplify high fidelity DNA than 10 kb in relatively short period of time (15-30 sec/kb).
- *n*Taq-multi HOT (Cat.# P725HC) can be used to perform general multiplex PCR and genotyping experiment related to genetic diagnostics.

II. Kit Components

Table 1. SuperiorScript III cDNA Kit components

Components	Volume (μ l)	
	S size	M size
SuperiorScript III RT (200 U/ μ l)	50	100
10X RT Buffer	1000	2000
25 mM MgCl ₂	500	1000
0.1 M DTT	500	1000
dNTP Mixture (10 mM each)	250	500
RNase Inhibitor	62.5	125
RNase H	50	100
Oligo dT (80 μ M)	50	100
Random hexamer (50 ng/ μ l)	250	500
Control RNA (10 ng/ μ l)	20	40
Control RT Primer MIX (10 pmol/ μ l each)	25	50
Sterile water (RNase free)	1000	2000

III. Procedure

1. RNA preparation

- Intact RNA is required for the synthesis of high-quality RNA in full-length. This kit is designed to be used with 1 pg~5 µg of total RNA or 1 pg~500 ng of mRNA.
- RNase inhibitor is included to prevent degradation of target RNA by ribonuclease contamination during RNA preparation.

2. RNase H digestion

- RNase H can improve PCR sensitivity especially on the long template after synthesizing first-strand cDNA by eliminating RNA template on cDNA:RNA hybrid. RNase H which is component of this kit can be properly used if necessary.
- The existence of RNase H while synthesizing the first-strand cDNA could decrease the yield of full-length cDNA.

3. First-Strand cDNA Synthesis

- ① Prepare one of the following RNA templates:
 - Total RNA: 1 pg-5 µg
 - mRNA : 1 pg-500 ng
- ② Prepare the following mixture in the 0.2 or 0.5 ml tube.

Components	Volume
Up to 5 µg total RNA	X µl
Primer*	1 µl
dNTP Mixture (10 mM each)	1 µl
Sterile water (RNase free)	Up to 10 µl
* Primer: 80 µM oligo dT, or 2 µM gene-specific Primer, 50 ng/µl random hexamer	

- ③ Incubate the tube for 5 min at 65°C and let it stand for 1 min on the ice. Centrifuge softly to collect the mixture in the tube and then add the following components.

Components	Volume
10X RT Buffer	2 µl
25 mM MgCl ₂	4 µl
0.1 M DTT	2 µl
RNase Inhibitor (40 U/µl)	1 µl
SuperiorScript III RT (200 U/µl)	1 µl

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- ④ Perform RT reaction.
 - For oligo dT or GSP : Incubate for 50 min at 50°C.
 - For random hexamer : Incubate for 10 min at 25°C, followed by 50 min at 50°C.
 - ⑤ Terminate the RT reaction by incubating the tube for 5 min at 85°C and then cool down on ice.
 - ⑥ Add 1 µl of RNase H and incubate the tube for 20 min at 37°C.

IV. Control Experiment Procedure

In vitro transcribed RNA (10 ng/µl) is provided as the control RNA in this kit. Control primer mix is designed to generate the RT-PCR product of 481 bp.

Proceed with the following protocol for the control RT reaction.

1. Dilute the control RNA with Sterile water(RNase free) to 1 ng/µl.
2. Prepare the following RNA/Primer mixture in the 0.2 or 0.5 ml tube.

Components	+ RT control	- RT control
Diluted control RNA (1 ng/µl)	1 µl	1 µl
Random hexamer (50 ng/µl)	1 µl	1 µl
dNTP Mixture (10 mM each)	1 µl	1 µl
Sterile water (RNase free)	7 µl	7 µl

3. Incubate the tube for 5 min at 65°C and let it stand for 1 min on ice. Centrifuge softly to collect the mixture in the tube and then add the following components.

Components	+ RT control	- RT control
10X RT Buffer	2 µl	2 µl
25 mM MgCl ₂	4 µl	4 µl
0.1 M DTT	2 µl	2 µl
RNase Inhibitor (40 U/µl)	1 µl	1 µl
SuperiorScript III RT (200 U/µl)	1 µl	-
Sterile water (RNase free)	-	1 µl

4. Incubate for 10 min at 25°C, followed by 50 min at 50°C.

5. Terminate the RT reaction by incubating the tube for 5 min at 85°C and then cool down on ice.
6. Add 1 µl of RNase H and incubate the tube for 20 min at 37°C.
7. Prepare the following PCR mixture for each control.

Components	Volume
2X TOPsimple™ DyeMIX-Tenuto (Cat.# P510)	10 µl
Control Primer MIX (10 pmol/µl each)	1 µl
cDNA from control RNA	1 µl
Sterile water (RNase free)	8 µl

8. Place the reaction mixture in preheated PCR machine at 94°C and perform the PCR reaction.

Initial Denaturation	95°C	2 min
Denaturation	95°C	30 sec
Annealing	60°C	30 sec
Elongation	72°C	1 min
Number of cycles 30 times		
Final Elongation	72°C	5 min

9. Load 5~10µl of each reaction mixture for agarose gel electrophoresis and analyze by ethidium bromide staining. Only the +RT control experiment should give rise to 481 bp band.