HyperScript™ First strand Synthesis Kit

GeneAll®

Ver. 1.02

Cat. No. 605-005 (50 reactions)

Storage at : -20 °C

Lot. No.

Expiration date :

Description

HyperScript™ First strand synthesis kit is ideally organized for synthesizing reaction of first strand cDNA from purified mRNA or total RNA. This kit provides all components for cDNA synthesis, including reverse transcriptase, RNase inhibitor, oligo dT, random hexamer, dNTPs and nuclease free water. From 1 pg to 2 μg of starting RNA can be applied as template and RNA-target up to 13 kb in length can be synthesized accurately. cDNA synthesis can be performed using either total RNA or poly(A)-selected RNA, primed with oligo dT, random primer or a gene specific primer.The included HyperScript™ Reverse Transcriptase is an engineered M-MLV Reverse Transcriptase with reduced RNase H activity and increased thermal stability. This enzyme can be used stably at temperatures up to 65°C for synthesizing of first-strand cDNA.

Components

HyperScript™ Reverse Transcriptase (200 U/μℓ)	1 vial (50 μℓ)
10X RTase reaction buffer	1 vial (500 μℓ)
0.1 M DTT	1 vial (250 μℓ)
10 mM dNTPs mix	1 vial (250 μℓ)
ZymAll™ RNase Inhibitor (40 U/μℓ)	1 vial (100 μℓ)
Oligo $(dT)_{20}$ (50 uM)	1 vial (50 μℓ)
Random hexamer (50 ng/μℓ)	1 vial (100 μℓ)
Nuclease free water	1 vial (1,000 μℓ)

Storage condition

Stable for 1 year at -20°C

Unit definition

- HyperScript™ Reverse Transcriptase
  One unit incorporates 1 nmol of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo$(dT)_{20}$ as template-primer.
- ZymAll™ RNase Inhibitor
  One unit is defined as the amount of Ribonuclease Inhibitor required to inhibit the activity of 5 ng of ribonuclease A by 50%.

Reagent to be supplied by user

Gene specific primer
RNase H

Procedure

(1) RNA template

The following 20-μl reaction mixture can be used for 1 pg – 2 μg of total RNA or 10 pg – 500 ng of poly(A) RNA.

(2) First-Strand synthesis of cDNA

1. Combine the followings in a nuclease-free tube.
   Mix gently and collect by brief centrifugation.

Components		Concentration	Amount
RNA (use one of the right column)	Total RNA	-	x μℓ (up to 2 μg)
	Poly(A) RNA	-	x μℓ (up to 500 ng)
Primer (use one of the right column)	Oligo dT	50 μM	1 μℓ
	Gene specific primer	2 μM	
	Random hexamer	50 ng/μℓ	
dNTPs		10 mM	1 μℓ
Nuclease free water		-	Adjust the mixture volume to 14 ul with water

2. Incubate at 65℃ for 5 min, then place on ice for at least 1 min.
3. Add the following components into the mix.

Components	Amount
10x RTase reaction buffer	2 μℓ
0.1 M DTT	2 μℓ
HyperScript™ Reverse Transcriptase (200 U/μℓ)	1 μℓ
ZymAll™ RNase Inhibitor	1 μℓ

* If the amount of starting RNA is less than 50 ng, the addition of RNase Inhibitor is essential.

4. Add each component, mix gently, and collect by brief centrifugation.
   Incubate as follows.

Primers	Reaction temperature	Incubation time
Oligo dT or Gene-specific primer	42~60℃ (recommend 55℃)	30~60 min
Random hexamer	25℃	5 min
	42~60℃ (recommend 55℃)	30~60 min

*The reaction temperature can be increased to the extent of 65℃ if the target gene has a somewhat intricate secondary structure.

5. Terminate the reaction by incubating at 85°C for 5 min. Chill on ice.
6. (Optional) To remove the residual RNA complementary to the cDNA, add 1~2 U of RNase H and incubate at 37°C for 20 min.

(3) Amplification of target cDNA

1. Select a DNA polymerase for PCR, depending on the length of the target DNA.

Length	DNA polymerase	Cat. No.
100 bp – 3 kb	Taq DNA polymerase	501-025
1 kb – 20 kb	α-Taq DNA polymerase	502-025
Hot start condition	HS-Taq DNA polymerase	531-025

2. Add cDNA mix to PCR reaction. The volume of added cDNA mix should not be over 10 % of the PCR reaction volume.