HyperScript™ Reverse Transcriptase

GeneAll®

Ver. 1.00

Cat. No. 601-100

Storage at : -20 °C

Lot. No.

Expiration date :

Description

HyperScript™ Reverse Transcriptase is an engineered M-MLV Reverse Transcriptase with reduced RNase H activity and increased thermal stability. The enzyme can be used to synthesize first-strand cDNA at temperatures up to 65°C. RNA target up to 13 kb can be detected with this enzyme. The amount of starting material can vary from 1 pg to 2 ug of total RNA. cDNA synthesis is performed using either total RNA or poly(A)-selected RNA, primed with oligo dT, random primer or a gene specific primer.

Components

HyperScript™ Reverse Transcriptase(200 U/㎕)	1 vial (50 ㎕)
10X RTase reaction buffer	1 vial (1000 ㎕)
0.1 M DTT	1 vial (250 ㎕)
10 mM dNTP mix	1 vial (250 ㎕)

Storage condition

Stable for 1 year at -20°C

Unit definition

One unit incorporates 1 nmol of dTTP into acid-precipitable material in 10 min at 37°C using poly(A) • oligo(dT)$_{25}$ as template-primer

Storage buffer

20 mM	Tris-HCl (pH 7.5)
100 mM	NaCl
0.1 mM	EDTA
1 mM	DTT
0.01 %	NP-40 (v/v)
50 %	glycerol (v/v)

Reagent to be supplied by user

RNase inhibitor

Primer : oligo dT, random primer or gene specific primer

RNase H

Procedure

1) RNA template

The following 20-㎕ reaction volume can be used for 1 pg – 2 ㎍ 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

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

2. Incubate at 65°C 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 ㎕
0.1 M DTT	2 ㎕
HyperScript™ Reverse Transcriptase(200 U/㎕)	1 ㎕
RNase inhibitor*	1 ㎕

* 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. Incubateion as follows.

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

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

5. Terminate the reaction 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.