HyperScript™ One-step RT-PCR premix

Ver. 1.00

Cat. No. 602-102

Storage at : -20 °C

Lot. No. M0201RPP

Expiration date : 2014.05.30

Description

Hyperscript™ one-step RT-PCR premix is a 2x premix ready to use for Reverse Transcriptase(RT) reaction and Polymerase Chain Reaction(PCR). The component of this premix is optimized for 20 ㎕ of reaction volume which is widely used for many downstream applications. This mix contains Hyperscript™ RTase and AmpONE™ HS-Taq DNA polymerase and both RT and PCR reactions are carried out successively in a single tube. HS-Taq DNA polymerase remains inactivated until RT reaction is completed, and it is turned on at high temperature of PCR cycle. HS-Taq polymerase can amplify the fragment up to 5 kb in length.

Hyperscript™ one-step RT-PCR premix contains all reaction components required for RT and PCR, such as reaction buffer, dNTPs, RNase inhibitor and stabilizer in addition to enzymes, except primers and templates.

Components

HyperScript™ one-step RT-PCR premix 96 tubes (8-tubes(0.2 ml) strip x 12ea with PCR tube rack)

Storage condition

Stable for 1 year at -20°C

Ingredients of Hyperscript™ one-step RT-PCR premix (based on 20 ㎕ reaction volume)

Hyperscript™ Reverse Transcriptase
HS-Taq DNA polymerase
dNTPs (mixture)
Reaction buffer (2.5 mM $MgCl_2$)
Stabilizer
RNase inhibitor

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) One step RT-PCR

1. Add the followings into 8-strip tubes

Components	Concentration	Amount
One step RT-PCR premix	2X	10 ㎕
Add the followings		
Total RNA		x ㎕ (up to 2 ㎍)
Forward Primer	10 pmol/㎕	1 ㎕
Reverse Primer	10 pmol/㎕	1.5 ㎕
DEPC treated water		Adjust the mixture volume to 20 ul with water

2. Add each component, mix gently and collect by brief centrifugation. Perform reaction as follows.

Step	Temp.	Time	Cycle No.
cDNA synthesis step	42 - 60°C (recommend- 55°C)	30 - 60 min	1 cycle
Pre-Denaturation	94°C	2 – 5 min	1 cycle
Denaturation	94°C	30 - 60 sec	25- 35 cycles
Annealing	X °C	30 - 60 sec	
Extension	72°C	1 min / kb	
Post-Extension	72°C	2-5 min	1 cycle

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