HyperScript™ 2X One-step RT-PCR Master Mix

Ver 3.1

Cat. No. 602-110 (0.5 ml x 2 tubes)

Storage at -20°C

Disclaimer

For research use only. Not for use in diagnostic or therapeutic procedures.

Description

HyperScript™ 2X One-step RT-PCR Master Mix is ready to use for reverse transcriptase (RT) reaction and polymerase chain reaction (PCR). This master mix contains HyperScript™ M-MLV reverse transcriptase (RNase H^-) and AmpONE™ HotStart *Taq* DNA Polymerase, and both reverse transcriptase and polymerase chain reactions are carried out successively in a single tube. Hot start *Taq* polymerase remains inactivated until reverse transcriptase reaction is completed, and it is turned on at high temperature of PCR cycle. Hot start *Taq* polymerase can amplify the fragment up to 1 kb in length. The reaction volume can be adjusted according to the experimental purpose. This master mix 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

Cat. No.	602-110
HyperScript™ 2X One-step RT-PCR Master Mix	0.5 ml x 2 tubes

Storage Conditions

Stable for 1 year at -20°C.

Ingredients of HyperScript™ 2X One-step RT-PCR Master Mix

Thermostable M-MLV reverse transcriptase (RNase H^-)
Hot start *Taq* DNA polymerase
Reaction buffer
dNTPs
Stabilizer
RNase inhibitor

Protocol

1. Prepare one of the following RNA template
   RNA template can be prepared using hand-made or commercial reagents. Pure RNA has a 1.8 - 2.1 of $A_{260/280}$ or 1.9 - 2.2 of $A_{260/230}$ ratio. If not, the result may not be good.

RNA template			
RNA	Total RNA	1 ng ~ 5 μg	- μl
	mRNA	1 ~ 250 ng	

2. One-step RT-PCR mixture

Components	Volume
HyperScript™ 2X One-step RT-PCR Master Mix	10 μl
Forward primer (5 pmol/μl)	1 μl
Reverse primer (5 pmol/μl)	1 μl
Template RNA	- μl
Add D.W. to	20 μl

3. One-step RT-PCR conditions

Step	Temp.	Time	Cycles
cDNA Synthesis	42~55°C (recommend 50°C)	30~60 min	1
Initial Denaturation	95°C	15 min *	1
Denaturation	95°C	20 sec	30~40
Annealing	**X**°C	30~60 sec	
Extension	72°C	30 sec	
Final Extension	72°C	2~5 min	1

* The chemical-modified HotStart enzyme requires a reactivation at 95°C for 15 min. If sufficient initial denaturation isn't performed, enzyme activity may be inhibited by chemicals that are not completely separated.