

Add RT-PCR SYBR Kit

Research Use Only

Product Code

71301

Component

1. 20x AddScript Enzyme Solution 50 μ l
2. 2.5x Buffer 0.5 ml

Storage Condition

Store at -20°C

Description

Add RT-PCR SYBR Kit provides sensitive and easy-to-use components which contain all the reagents for first strand cDNA synthesis and PCR reaction in one-tube reaction, and is designed for high sensitivity and specificity on various real-time instruments.

Especially, thermostable MMLV RTase (RNase H-), hot-start Taq DNA Polymerase and RNase Inhibitor are included in 20x AddScript Enzyme Solution.

Quality Control

The performance of Add RT-PCR SYBR Kit is tested in a RT and PCR one-tube reaction using human total RNA with specific primers. The sensitivity of the kit is verified by the detection of GAPDH and Actin transcript in 10 pg total RNA after 30 cycles.

Storage and Stability

Add RT-PCR SYBR Kit is stable for 2 year when stored in a constant temperature freezer at less than -20°C.

Nucleic Acid Amplification Protocol

1. Add the following components to a thin-walled PCR tube:

Nuclease-Free H ₂ O	x µl
2.5x Buffer	8.0 µl
Forward primer (10 µM)	0.25~2.0 µl
Reverse primer (10 µM)	0.25~2.0 µl
(Optional) 50x ROX dye	x µl
RNA template	x µl
20x AddScript Enzyme Solution	1.0 µl
Total reaction volume	20 µl

* Recommendation for template RNA concentration in a 20 µl reaction volume

1) total RNA: 100 fg ~ 1 µg

2) mRNA: 10 fg ~ 1 µg

2. PCR cycling

cDNA synthesis	50°C, 20 min
Initial denaturation	95°C, 10 min
PCR cycling (40 cycles)	95°C, 15 sec 60°C, 1 min
Melting analysis	60°C → 90°C

[Note] 50x ROX dye

ROX dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments which are compatible with that option.

50x ROX is a 25 µM concentration. Use the following table to determine the amount of ROX to use with a particular instrument.

Instrument	Final ROX concentration
AB 7000, 7300, 7700, 7900HT, 7900 Fast, StepOne and StepOnePlus	500 nM
AB 7500, 7500 Fast, Stratagene Mx3000P, Mx3005P and Mx4000	50 nM