AddScript RT-PCR Kit

Research Use Only

Product Code

23601

Component

- 1. 20x AddScript Enzyme Solution 50 µl
- 2. 2.5x Buffer 0.5 ml
- 3. Nuclease-free H₂0 1.0 ml

Storage Condition

Store at -20°C

Description

AddScript RT-PCR 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 continuously.

Especially, thermostable MMLV RTase (RNase H-), hot-start Taq DNA Polymerase and RNase Inhibitor are included in 20x AddScript Enzyme Solution. The loading dye and sediment for electrophoresis and dNTP mixture are also included in 2.5x Buffer.

Quality Control

The performance of AddScript RT-PCR 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

AddScript cDNA Synthesis Kit is stable for 1 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 0	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
RNA template	хμΙ
20x AddScript Enzyme Solution	1.0 μΙ
Total reaction volume	20 μΙ

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

2. Temperature cycling Protocol

cDNA synthesis	50°C, 30 min
Initial denaturation	95℃, 10 min
PCR cycling (30 – 40 cycles)	95°C, 15 – 30 sec
	55 - 65°C, 15 – 30 sec
	72℃, 1 min
Final extension	72℃, 5 min
Hold	12℃, ∞

¹⁾ total RNA: 100 fg \sim 1 μg

²⁾ mRNA: 10 fg ~ 1 μg