AddScript cDNA Synthesis Kit

Research Use Only

Product Code

22701

Component

- 1. 20x AddScript Enzyme Solution 50 µl
- 2. 2x Reaction Buffer 0.5 ml
- 3. 10mM dNTP Mixture (2.5mM each) 0.1 ml
- 4. 10x oligo dT₂₀ 0.1 ml
- 5. 10x random hexamer 0.1 ml
- 6. Nuclease-free H₂0 1.0 ml

Storage Condition

Store at -20°C

Description

AddScript cDNA Synthesis Kit provides sensitive and easy-to-use components which contain all the reagents for first strand cDNA synthesis.

Especially, thermostable MMLV RTase (RNase H-) and RNase Inhibitor are included in 20x AddScript Enzyme Solution. This kit can be also applied to real-time PCR by fluorescent intercalating dye like SYBR Green I® and TaqMan® Probe.

Quality Control

The performance of AddScript cDNA Synthesis Kit is tested in an RT reaction using human total RNA with oligo dT_{20} and random hexamer each. 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 years when stored in a constant temperature freezer at less than -20°C.

Reaction Assembly

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

Nuclease-Free H ₂ 0	x μl
2x Reaction Buffer	10 μΙ
10mM dNTP Mixture	2.0 μΙ
10x oligo dT ₂₀ [or 10x random hexamer]	2.0 μΙ
RNA template	x μl
20x AddScript Enzyme Solution	1.0 μΙ
Total reaction volume	20 μΙ

 $^{^{\}star}$ Recommendation for template RNA concentration in a 20 μl reaction volume

2. Temperature cycling Protocol

Priming	25°C, 10 min
Reverse transcription	50°C, 60 min
RT inactivation	80°C, 5 min
Hold	12℃, ∞

Recommendation for downstream PCR

For downstream PCR amplification, the volume of cDNA product should not exceed 1/5 of the PCR reaction volume, typically 1 \sim 4 μ l in 20 μ l PCR reaction.

¹⁾ total RNA: 100 fg ~ 1 μg

²⁾ mRNA: 10 fg \sim 1 μg