# **AddScript RT Master** (2x Conc.)

**Research Use Only** 

#### **Product Code**

22101

#### Component

1. AddScript RT Master (2x conc.) 1.0 ml

### **Storage Condition**

Store at -20°C

## Description

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

Especially, thermostable MMLV RTase (RNase H-), reaction buffer, dNTP mixture, and enzyme stabilizer are included in 2x AddScript RT Master.

### **Usage Information**

- The reaction temperature for cDNA synthesis is 50℃.
- The reaction time for cDNA synthesis is 60 min.

# **Quality Control**

The performance of AddScript RT Master (2x) 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 RT Master (2x) is stable for 2 years when stored in a constant temperature freezer at less than  $-20^{\circ}$ C.

### **Reaction Assembly**

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

Nuclease-free D.W	x μl
2x AddScript RT Master	10 μΙ
10mM dNTP Mixture (Not provided)	2.0 μΙ
50~100 pmoles/µl oligo dT <sub>20</sub> (random hexamer) or Gene specific primer (10~20 pmoles/µl)	1.0 μΙ
RNA template	x μl
RNase Inhibitor (Optional)	x μl
Total reaction volume	20 μΙ

<sup>\*</sup> Recommendation for template RNA concentration in a 20 µl reaction volume

# 2. Temperature cycling Protocol

Priming	25℃, 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  $\mu$ l in 20  $\mu$ l PCR reaction.

<sup>1)</sup> total RNA: 100 fg  $\sim$  1  $\mu g$ 

<sup>2)</sup> mRNA: 10 fg  $\sim$  1  $\mu$ g