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

**Research Use Only** 

#### **Product Code**

24701

#### Component

1. AddScript RT-PCR Master 1ml

### **Storage Condition**

Store at -20°C

#### Description

AddScript RT-PCR Master 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, loading dye and sediment for electrophoresis and dNTP mixture are included in 2x Master Mix.

## **Quality Control**

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

## **Nucleic Acid Amplification Protocol**

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

| Nuclease-free D.W          | х μΙ        |
|----------------------------|-------------|
| 2x AddScript RT-PCR Master | 10.0 μΙ     |
| Forward primer (10 µM)     | 0.25~2.0 μl |
| Reverse primer (10 µM)     | 0.25~2.0 μl |
| RNA template               | х μΙ        |
| Total reaction volume      | 20 μΙ       |

<sup>\*</sup> 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<br>(30– 40 cycles) | 95℃, 15 – 30 sec       |
|                                | 55 - 65°C, 15 – 30 sec |
|                                | 72℃, 1 min             |
| Final Extension                | 72℃, 5 min             |
| Hold                           | 12℃, ∞                 |

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

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