## AddScript Reverse Transcriptase

## Product Code

21001

## Component

1. 20x AddScript Reverse Transcriptase $50 \mu \mathrm{l}$
2. $2 x$ Reaction Buffer 0.5 ml

## Storage Condition

Store at $-20^{\circ} \mathrm{C}$

## Description

AddScript Reverse Transcriptase is a mutant of MMLV Reverse Transcriptase with reduced RNase H activity and increased thermal stability.

## Usage Information

- The reaction temperature for cDNA synthesis is $50^{\circ} \mathrm{C}$.
- The reaction time for cDNA synthesis is 60 min .


## Quality Control

The performance of AddScript Reverse Transcriptase is tested in an RT reaction using human total RNA with oligo $\mathrm{dT}_{20}$ 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 Reverse Transcriptase is stable for 2 years when stored in a constant temperature freezer at less than $-20^{\circ} \mathrm{C}$.

## Reaction Assembly

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

| Nuclease-free D.W | $\times \mu \mathrm{l}$ |
| :--- | :--- |
| $2 \times$ Reaction Buffer | $10 \mu \mathrm{l}$ |
| 10 mM dNTP Mixture (Not provided) | $2.0 \mu \mathrm{l}$ |
| $50 \sim 100$ pmoles $/ \mu \mathrm{l}$ oligo $\mathrm{dT}_{20}$ (random hexamer) | $1.0 \mu \mathrm{l}$ |
| or Gene specific primer (10~20 pmoles/ $\mu \mathrm{l}$ ) | $\mathrm{x} \mu \mathrm{l}$ |
| RNA template | $\mathrm{x} \mathrm{\mu l}$ |
| RNase Inhibitor (Optional) | $1.0 \mu \mathrm{l}$ |
| 20x AddScript Reverse Transcriptase | $20 \mu \mathrm{l}$ |
| Total reaction volume |  |

* Recommendation for template RNA concentration in a $20 \mu$ l reaction volume

1) total RNA: $100 \mathrm{fg} \sim 1 \mu \mathrm{~g}$
2) mRNA: $10 \mathrm{fg} \sim 1 \mu \mathrm{~g}$
2. Temperature cycling Protocol

| Priming | $25^{\circ} \mathrm{C}, 10 \mathrm{~min}$ |
| :--- | :--- |
| Reverse transcription | $50^{\circ} \mathrm{C}, 60 \mathrm{~min}$ |
| RT inactivation | $80^{\circ} \mathrm{C}, 5 \mathrm{~min}$ |
| Hold | $12^{\circ} \mathrm{C}, \infty$ |

## Recommendation for downstream PCR

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

