# *Rocketscript ™ Reverse Transcriptase*

**[Cat. No.]**
**E-3141**

**[Lot No.]**

**[Conc]**
**200 U/μl**

**Exp,Date:**

## ● Introduction :

*RocketScript* Reverse Transcriptase is a new M-MLV originated Reverse Transcriptase optimized to synthesize first-strand cDNA from purified Poly(A) or total RNA template. RNA targets from 100 bp to > 10 kb can be detected with the RocketScript RT.

The amount of starting material can vary from 1 pg to > 1 ug of total RNA. RocketScript Reverse Transcriptase is a version of M-MLV RT that has been engineered to provide increased thermal stability.

The enzyme can be used to synthesize cDNA at a temperature range of 42 - 70℃, providing increased specificity , higher yields of cDNA, and more full-length product than other reverse transcriptases.

## ● Principle:

*RocketScript* Reverse Transcriptase is genetically engineered, thermal stable M-MLV with enhanced thermal stability and outstanding processivity. The enzyme also features increased specificity and improved efficiency allowing efficient reverse transcription of RNA molecules with complex secondary structures

## ● Contents

| Component | Quantity |
|---|---|
| *RocketScript Reverse Transcriptase* (10,000 U) | 50 μl |
| 5 X Reaction Buffer | 0.5 ml |
| 100 mM DTT | 0.3 ml |
| 10 mM dNTP | 0.2 ml |
| RNase Inhibitor(100ng/ μl) | 50 μl |

## ● Protocol :

**A. First-strand cDNA synthesis [20 μl reaction volume ]**

Step 1 (No Incubation)

Total RNA (1 ug) or RNA (5 – 100 ng) X μl
Oligo dT or random primer (10 – 100 pmoles) X μl
water( RNase- and DNase– free) Variable volume

Step 2

5 X RocketScript Reaction Buffer 4 μl
100 mM DTT 2 μl
10 mM dNTP (Variable volume) or 2 μl
RNase inhibitor (50 – 100 ng) X μl
RocketScript Reverse Transcriptase (200 U/μl) 1 μl
DEPC-D.W Variable volume
Total (Step1 + Step 2) 20 μl

**B. Reverse Transcription protocol**

1. Cyclic Reverse Transcription (CRT)

| Step | Temperature | | | | Time | Cycle |
|---|---|---|---|---|---|---|
| | dN6 | dN12 | DT20 | Specific Primer | | |
| Primer annealing | 15℃ | 30℃ | 37℃ | TM Value | 10~30 sec | 12 |
| cDNA synthesis | 50℃ | | | | 4 min | |
| Melting secondary structure & cDNA synthesis | 55℃ | | | | 30 sec | |
| Heat inactivation | 95℃ | | | | 5 min | 1 |

**Note : Store the buffer by low aliquots at -20℃ to minimize degradation of the DTT**

2. Fixed Temperature Reverse Transcription (FTRT)

| Step | Temperature | | | | Time |
|---|---|---|---|---|---|
| | dN6 | dN12 | dT20 | Specific Primer | |
| Primer annealing | 15℃ | 30℃ | 37℃ | TM Value | 1 min |
| cDNA synthesis | 42~70℃ | | | | 10~60 min |
| Heat inactivation | 95℃ | | | | 5 min |

Note:. Or Perform cDNA synthesis reaction as follow 50℃ 60min(cDNA synthesis), 94℃,5min(RTase Inactivation). RT reaction temperature should be selected to fit the Tm value of Primers

● **Experimental Data**

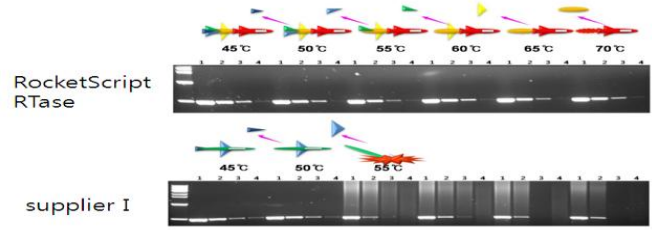


**Figure 1. Amplification results of *RocketScript* Reverse Transcriptase using myc compared with supplier I**
Reverse transcription condition: Incubation at each temperature 45, /50,/ 55, /60, /65, /70 °C for 1 hr, inactivation at 95°C for 5 min
Primer set: human myc 495bp set
Lane M : 1 kb DNA Ladder
Lane 1: 10 ng Human total RNA from HeLa cell Lane 2: 1 ng Human total RNA from HeLa cell
Lane 3: 100pg Human total RNA from HeLa cell Lane 4: 10 pg Human total RNA from HeLa cell

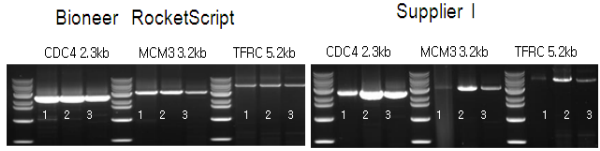


**Figure 2. Comparison of long kb amplification between RocketScript RTase and RT enzyme from supplier I**
RT reaction conditions were performed according to each manufacturer's recommendation. All cDNAs were amplified with AccuPower ®Hotstart PCR Premix (K-5050) from Bioneer
Note supplier I shows inhibition in high input concentration of total RNA
Lane 1: 1 µg Human total RNA from HeLa cell Lane 2: 100 ng Human total RNA from HeLa cell
Lane 3: 10 ng Human total RNA from HeLa cell


**Figure 3. Comparison of amplification quality between RocketScript RTase and other company's RTase**
Sensitivity test. Target gene Expression Level.
Lane M : 1 kb DNA Ladder
Lane 1: 100 ng Human total RNA from HeLa cell Lane 2: 10 ng Human total RNA from HeLa cell
Lane 3: 1 ng Human total RNA from HeLa cell Lane 4: 100 pg Human total RNA from HeLa cell

## ● Ordering Information

| Cat. No. | Description |
|---|---|
| E-3141 | *RocketScript* reverse Transcriptase, 10,000 U (50 Rxn) |
| E-3142 | *RocketScript* reverse Transcriptase, 50,000 U (250 Rxn) |