# CycleScript Reverse Transcriptase™

**[Cat. No.]**
**E-3131**
**E-3132**

**[Lot No.]**
**0701**

**[Concentration]**
**200 units/uL**

● **Description :** Bioneer's CycleScript Reverse Transcriptase is extremely pure and stable enzyme which is engineered for improved performance over a broad range of temperature from 37 to 55. The enzyme can be used over the broadest temperature range with high efficiency.
Thus, it is the most suitable enzyme for Cyclic Reverse Transcription (Cyclic RT reaction) which performs homogeneous primer-annealing at low temperature as well as reverse transcription with high sensitivity at high temperature, which even complex RNA secondary structure is resolved.
The Cyclic RT reaction is performed accordingly this protocol: Step 1 is performed at 12~40℃, at which short primer is easily annealed and extended. And then, the Step 2 is performed at 47~48℃ (optionally) and the Step 3 is performed at higher temperature 50~55℃at which reverse transcription is obstructed owing to RNA secondary structure. Finally, reverse transcription is also occurred. The above Cyclic RT reaction is repeated during the reaction (patent pending).

● **Quality Assurance :** The activity of DNase and RNase is not detected after incubation of 1 ug of DNA and RNA with 200 units of CycleScript Reverse Transcriptase for 3 hours at 37 ~ 42.

● **Source :** M-MLV Reverse Transcriptase is isolated from a *E.coli* strain containing a recombinant clone.

● **Application**
First-strand cDNA synthesis from RNA, Sequencing of single-stranded and double-stranded DNA or RNA. Random priming reaction, Library construction, Probe labeling, RT-PCR, mRNA 5'-end Mapping by Primer Extension Analysis

● **Supplied with Enzyme**
**CycleScript (200unit/ul) 10,000 U ; Natural cap**
**5 X Reaction Buffer (0.4mL) ; Red cap**
**100mM DTT (0.2mL) ; Green cap**
**10mM dNTP(0.2mL) : Black cap**

● **Note :** Store the buffer by low aliquots at -20 to minimize degradation of the DTT
For research use only. Not for use in diagnostic or therapeutic procedures.

● **Storage condition :** 20 mM Tris-Cl (pH7.6), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 % NP-40, 50mM $(NH_4)_2SO_4$, 50 % Glycerol, store at –20

● **Unit Definition :** One unit is defined as the enzyme activity which incorporates 1 nmole dTTP into acid-insoluble products in 10 min at 37 with poly(A) template RNA and oligo (dT) primer.

● **Features and Benefits :**

**- High Stable**
The improved stability of CycleScript Reverse Transcriptase supports accurate and high yields in cDNA synthesis. As CycleScript Reverse Transcriptase is manufactured in optimized manufacturing-process, it is guaranteed free from RNase, DNase, and Proteinase.
**- Improved performance over a broad range of cDNA synthesis temperature**
CycleScript Reverse Transcriptase is engineered for improved performance over a broad range of cDNA synthesis synthesis temperature from 37 to 55℃. It is exceptionally unique product having superior reactivity and thermo-stability. It is also applicable to both Cyclic RT reaction and conventional RT reaction.
**- Superior Affinity & good cDNA yield**
Cyclic RT is carried out by annealing with primers with a broad range of Tm-value at low temperature and by dissolving secondary RNA structure at high temperature. This shows an excellent affinity of primer and template, and gives the ability to read through and synthesized full-length cDNA even complex RNA secondary structure without adjusting temperature or reaction conditions.
**- Simple & Speedy**
In case achieving Cyclic RT reaction, the pre-incubation process for primer and RNA template is unnecessary. It makes it to simplify experiment method and to shorten reaction time.

- *Procedure*

**- first-strand cDNA synthesis [ 20 uL reaction volume ]**

Step 1
**Total RNA or mRNA**
→ 1 ug total RNA or 5 ng-100 ng mRNA
**Oligo dT (or random primer)**
→ 10 – 100 pmoles
**DEPC water( RNase - and DNase – free** )
→ variable volume

Step 2

| | |
|---|---|
| 5X CycleScript reaction buffer | 4 uL |
| 100mM DTT | 2 uL |
| 10mM dNTP (variable volume) | 2 uL |
| RNase inhibitor | 20 units |
| CycleScript(200unit/ul) | 1 uL |
| Total (Step1 + Step 2) | 20 uL |

*** ➔ Denature RNA and primer for 10 min at 65 °C**
*** ➔ Immediately cool on ice**
***Note. For Cyclic RT, this(*) step can be omissible.**

**➔ [Option 1: Cyclic RT, CRT]**
**or [Option 2: Fixed Temperature RT, FTRT]**

| [Option 1: CRT] | | |
|---|---|---|
| **Temperature** | **Time** | |
| 15 ~ 37℃ | 30 sec | Repeat 12 times |
| 42 ~ 48℃ | 4 min | |
| 55℃(Optional) | 30 sec | |
| Deactivation | | |
| 90℃ | 5 min | |

| [Option 2: FTRT] | |
|---|---|
| **Temperature** | **Time** |
| 37 – 55℃ | 1 hr |
| Deactivation | |
| 95℃ | 5 min |

- *Principle*

The conventional RT reaction at 42°C has two drawbacks: (i) Change of RT reaction temperature is needed to perform cDNA synthesis because the common binding primer (e.g. oligo dT, random primer) annealed to template RNA is rather short and has Tm value from 15 to 40℃. (ii) A complex secondary RNA structure obstructs cDNA synthesis from RNA at 42°C.
CycleScript Reverse Transcriptase from Bioneer solved them, with which makes it to perform Cyclic RT reaction which performs homogeneous primer-annealing at low temperature as well as reverse transcription with high sensitivity at high temperature, which even complex RNA secondary structure is resolved.

- *Ordering Information*

| Cat. No. | Products |
|---|---|
| EB-1003 | 5 X CycleScript reaction buffer (0.4mL) |
| K-2041 | 96tube, AccuPower® RT premix 0.2ml tube 20ul reaction |
| K-2055 | 96tube, AccuPower® RT-PCR premix 0.2ml tube 20ul reaction |
| K-3033 | AccuPrep® Viral RNA Extraction Kit 100 reaction |
| K-3060 | Viral RNA *PrepMate*™ 100 reaction |
| K-3070 | Blood RNA *PrepMate*™ 100 reaction |
| K-3080 | Tissue RNA *PrepMate*™ 100 reaction |
| A-2040 | MyGene® 96 Gradient Thermal Block |
| K-2044 | AccuPower ™ CycleScript RT Premix (dT)20 |
| K-2045 | AccuPower ™ CycleScript RT Premix (dN)12 |
| K-2046 | AccuPower ™ CycleScript RT Premix (dN)6 |