molecular designs

PRODUCT INFORMATION

Catalog No.: G237

Product Name: **EasyScript Plus™ Reverse Transcriptase**

Size:	**100 rxn**
Concentration:	**200 U/µl**

Description: **EasyScript Plus™ Reverse Transcriptase** is based on Moloney-Murine Leukemia Virus Reverse Transcriptase with genetic modifications to abolish RNase H activity to achieve thermal stability. The **EasyScript Plus™ Reverse Transcriptase** is engineered to work under high temperatures (50°C-55°C), which can further facilitate to resolve the secondary structures and high GC problems of RNA. RNaseOFF Ribonuclease Inhibitor formulated in the enzyme system further imprive the overall performance. Due to these features, full-length cDNA can be synthesized from RNA templates that are up to 12 kb.

Application:
-RT-PCR
-Real Time RT-PCR
-cDNA library
- SAGE
-3' or 5' RACE

Kit Components:

Component	Volume
EasyScript Plus™ RTase (200U/µl)	100 rxn/100µl
5x RT buffer	400 µl

Enzyme Storage Buffer : 50mM Tris-HCl (pH 8.3), 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT,
0.1% (v/v) Triton X-100, and 50% (v/v) glycerol.

5x RT Reaction Buffer: 250mM Tris HCl (pH 8.3), 375mM KCl, 15mM MgCl2, and trace amount of cDNA synthesis enhancer.

Storage Conditions: Store all components at -20°C.

- 2X qPCR Universal Green MasterMix qMX-Green
- 2X qPCR Universal TaqProbe MasterMix qMX-TaqM
- 100bp DNA Ladder M107
- 1Kb DNA Ladder II M108

- DNA SafeStain C138
- Standard-Agarose A113

General Protocol

RT-PCR reactions should be assembled in a RNA-free environment. The use of clean pipettes designated for PCR and aerosol resistant barrier tips are recommended.

1. Thaw template RNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.

2. Prepare the following reaction mixture in a tube on ice:

Component	Volume
5X RT Buffer	4 µl
dNTP	1 µl
Primers	1 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
EasyScript® Plus RTase	1 µl
Nuclease-free H2O	up to 20 µl

3. Mix thoroughly and carefully by vortexing for 3 -5 seconds. Centrifuge briefly to collect the contents of the tube, and incubate at 25°C for 5 minutes if random primer is used. Omit this step if Oligo(dT) primer or sequence-specific primer are used.

4. Incubate at 50°C -55°C for 20 minutes.

5. Stop the reaction by heating at 85°C for 5 minutes. Chill on ice. The synthesized first-strand cDNA can be used directly for downstream applications or store at -20ºC for further use.

Notes:

1. Isolation of poly(A)$^+$RNA from total RNA is not mandatory. However, doing so may improve the yield and purity of the final product.

2. In most cases, cDNA synthesized with this enzyme can be directly used as a template for most polymerase chain reactions (PCR), without further purification. Generally, dilute the final reaction mix for 10 times with water. Use 1 – 2 µl of the diluted reaction mix for each PCR reaction.

3. RNA sample must be free of contaminating genomic DNA.

4. Unlike the oligo(dT) priming, which usually requires no optimization, the ratio of a random primer to RNA is critical in terms of the average length of cDNA synthesized in the reaction. Increasing the ratio of random primer/RNA will result in higher yield of shorter (~500bp) cDNA, whereas decreasing this ratio will produce longer products.

5. For longer transcripts >9 kb, yields can be increased by incubating at 50-55°C up to 60 minutes.

Note: This Product Is For Research Use Only