

HiScript I Reverse Transcriptase

Cat. No. : AM0670
Concentration : 200 Units/ μ l
Volume : 50 μ l
Storage : -20 °C

Description

HiScript I Reverse Transcriptase is a multiple-point mutated version of M-MLV RT. The enzyme is purified from *E. coli* containing the mutated pol. gene of Moloney Murine Leukemia Virus. The enzyme can be used to synthesize first-strand cDNA at higher temperatures than M-MLV RT, providing increased specificity, higher yield of cDNA.

Form

20 mM Tris-HCl (pH 7.8)
100 mM NaCl
0.1 mM EDTA
1 mM DTT
50 % glycerol

Components

Script RT
5X First-Strand Buffer
(250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM MgCl₂)
0.1 M DTT

Unit Definition

One unit incorporates 1 nmole of dTTP into acid precipitable material in 10 mins at 37°C using poly(A)-oligo(dT) as template primer.

Standard protocol for First-Strand cDNA synthesis

- Add the following components to a microtube.
Oligo dT primer 50 pmole /Random primer 50 pmole

/ Gene specific primer 2 pmole
dNTPs Mixture (10 mM each)... 1 μ l
Template RNA
(total RNA \leq 5 μ g or mRNA \leq 1 μ g)
Sterile, distilled water to 12 μ l

- Heat at 65°C for 5 mins, and cool immediately on ice.
Collect the contents of the tube by brief centrifugation.
- Prepare the reaction mixture by combining the following reagents to a total volume 20 μ l .
Template RNA / Primer mixture ... 12 μ l
5X First-Strand Buffer 4 μ l
0.1 M DTT 2 μ l
RNase Inhibitor (optional)* 1 μ l
HiScript I Reverse Transcriptase ... 1 μ l
- Mix gently and spin down .
- Perform the reaction under the following condition
30°C 10 mins* \rightarrow 42 (~ 48) °C** 30~60 mins.

- Heat at 70°C for 15 mins.

* This step is required for random primer.

** It is generally recommended to perform the RT reaction at 42°C with this enzyme . However , if the reverse primer for PCR is also used as a RT primer , non-specific products may be amplified due to mispriming . In such a case , it is recommended to perform the RT reaction at 48°C .

PCR

Use only 2 μ l of the First-Strand reaction for PCR .

- Add the following components to a PCR tube .
10X PCR Buffer..... 5 μ l
10 mM dNTPs Mixture..... 1 μ l
10 μ M Forward primer 1 μ l
10 μ M Reverse primer 1 μ l
5U/ μ l Taq DNA polymerase ... 0.4 μ l
The First-Strand reactant..... 2 μ l
Autoclaved , distilled water to 50 μ l
- Mix gently and spin down .
- perform 20 to 40 cycles of PCR .

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