Invitrogen
life technologies

ThermoScript™ Reverse Transcriptase

Cat. No. 12236-014 **Size: 375 Units**
Cat. No. 12236-022 **Size: 1,500 Units**

Conc. 15 U/µl **Store at -20°C in a non-frost free freezer**

Description

ThermoScript™ Reverse Transcriptase (RT) is an avian reverse transcriptase that has been engineered for reduced RNase H activity and higher thermal stability (1). It produces higher yields of cDNA and more full-length cDNA transcripts than AMV RT. ThermoScript™ RT can generate cDNA transcripts from 100 bp to >12 kb at temperatures ranging from 50°C to 65°C.

Component	375-U Kit	1,500-U Kit
ThermoScript™ RT (15 U/µl)	25 µl	100 µl
5X cDNA Synthesis Buffer	500 µl	2 × 500 µl
0.1 M DTT	100 µl	100 µl

Unit Definition

One unit incorporates 1 nmol of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo(dT)$_{25}$ as template-primer .

Storage Buffer

200 mM KPO_4 (pH 7.1), 0.1 mM EDTA, 1 mM DTT, 0.05% (v/v) Triton® X-100, 50% (v/v) glycerol, stabilizers

5X cDNA Synthesis Buffer

250 mM Tris acetate (pH 8.4 at room temperature), 375 mM potassium acetate, 40 mM magnesium acetate

Storage and Handling

Store ThermoScript™ RT at -20°C in a non-frost-free freezer. Stability may be extended by storing at -70°C. Store the 5X cDNA Synthesis Buffer and 0.1 M DTT at -20°C. Thaw the solutions at room temperature just prior to use and refreeze immediately.

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First-Strand cDNA Synthesis Using ThermoScript™ RT

1. Add the following components to a nuclease-free microcentrifuge tube:

Oligo $(dT)_{20}$ (50 µM) *or* 200–500 ng $(dT)_{12–18}$ *or* 50–250 ng random primers *or* 10–20 pmole gene-specific primer	1 µl
10 pg to 5 µg total RNA *or* 10 pg to 500 ng of mRNA	x µl
10 mM dNTP Mix	2 µl
Sterile, distilled water	to 12 µl

2. Incubate mixture at 65°C for 5 min and then place on ice (optional). Collect the contents of the tube by brief centrifugation and add:

5X cDNA Synthesis Buffer	4 µl
0.1 M DTT	1 µl
RNaseOUT™ (40 units/µl) (optional)*	1 µl
Sterile, distilled water	1 µl
ThermoScript™ RT (15 U/µl)**	1 µl

*RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777-019) is required if using <50 ng starting RNA.

**If less than 1 ng of RNA is used, reduce the amount of ThermoScript™ RT in the reaction to 0.5 µl (7.5 units) and increase the amount of sterile, distilled water to 1.5 µl/reaction (2).

3. If you are using random primers, incubate tube at 25°C for 10 min.
4. Mix contents of the tube gently and incubate at 50°C for 30-60 min. (If you are using oligo$(dT)_{20}$ or gene-specific primers, you can incubate at 50–65°C.)
5. Terminate the reaction by heating at 85°C for 5 min.

Note that amplification of PCR targets >1 kb may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µl (2 units) of *E. coli* RNase H and incubate at 37°C for 20 min.

PCR

The following is intended as a guideline and starting point when using first-strand cDNA in PCR with *Taq* DNA polymerase. The optimal concentration of Mg^{++} will vary depending on the template and primer pair.
Use only 10% of the first-strand reaction for PCR. Higher volumes may not increase amplification and may result in decreased amounts of PCR product.

1. Add the following to a PCR tube:

10X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5 µl
50 mM $MgCl_2$	1.5 µl
10 mM dNTP Mix	1 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
Taq DNA polymerase (5 U/µl)	0.4 µl
cDNA from first-strand reaction	2 µl
autoclaved, distilled water	to 50 µl

2. Mix gently and layer with 1–2 drops (~50 µl) of silicone oil. *(Note: Silicone oil is unnecessary in thermal cyclers equipped with a heated lid.)*
3. Heat reaction to 94°C for 2 min to denature.
4. Perform 15 to 40 cycles of PCR. Use the recommended annealing and extension conditions for your *Taq* DNA polymerase.

Quality Control

This product has passed the following quality control assays: SDS-polyacrylamide gel analysis for purity; functional absence of endodeoxyribonuclease, 3′ and 5′ exodeoxyribonuclease, and ribonuclease activities; yield and length of cDNA product.

References

1. Schwabe, W., Lee, J.E., Nathan, M., Xu, R.H., Sitaraman, K., Smith, M., Potter, R.J., Rosenthal, K., Rashtchian, A., and Gerard, G.F. (1998) Focus® 20, 30.
2. Huang, L., Lee, J., Sitaraman, K., Gallego, A., and Rashtchian, A. (2000) Focus® 22, 3.

Limited Use Label License No. 4: Products for PCR which do not include any rights to perform PCR

Limited Label License No. 13: ThermoScript™ Enzyme