



## 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

	<u>375-U Kit</u>	<u>1,500-U Kit</u>
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)<sub>25</sub> as template-primer.

### Storage Buffer

200 mM KPO<sub>4</sub> (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.

Part no. 12236.pps

Rev. date: 26 Sep 2003

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

**First-Strand cDNA Synthesis Using ThermoScript™ RT**

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

Oligo (dT) <sub>20</sub> (50 μM) <i>or</i>	1 μl
200–500 ng (dT) <sub>12-18</sub> <i>or</i>	
50–250 ng random primers <i>or</i>	
10–20 pmole gene-specific primer	
10 pg to 5 μg total RNA <i>or</i>	x μl
10 pg to 500 ng of mRNA	
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)<sub>20</sub> 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<sup>++</sup> 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 <sub>2</sub>	1.5 µl
10 mM dNTP Mix	1 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
<i>Taq</i> DNA polymerase (5 U/µl)	0.4 µl
cDNA from first-strand reaction	2 µl
autoclaved, distilled water	to 50 µl

- 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.*)
- Heat reaction to 94°C for 2 min to denature.
- 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.

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