EURx® MOLECULAR BIOLOGY PRODUCTS

Tth Reverse Transcriptase

(Thermus thermophilus)

Cat. No.	size
E1374-01	100 units
E1374-02	500 units

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble form in 10 min at 50°C.

Storage Conditions: Store at -20°C.

References:

1. *Mulder, I. et al. (1994) Journal of Clinical Microbiology 32, 292-300.*
2. *Wang, A. M., Doyle, M.V. and Mark, D.F. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 9717-9721.*
3. *Myers, T. W., Gelfanld, D. H. (1991) Biochemistry 30, 7661-7666.*
4. *Kather, H. L., Schwartz, I. (1994) Biotechniques 16, 84-92.*
5. *Poddar, S. K., Sawyer, M. H., Connor, J. D. (1998) J. Med. Microbiol. 47, 1131-1135.*

***Thermus thermopilus* Reverse Transcriptase catalyzes the reverse transcription of RNA to cDNA at elevated temperatures in the presence of Mn^{2+} and catalyzes polymerization of DNA in the presence of Mg^{2+}.**

Description:

- Suitable for high temperature synthesis of DNA (2).
- Synthesizes cDNA from RNA template (3).
- Can reverse transcribe at elevated temperatures (3).
- Minimizes problems with strong secondary structure of RNA.
- Used for efficient PCR of DNA, containing problematic secondary structures.
- Applicable to RT-PCR; the same enzyme is used for both reverse transcription and following amplification of obtained cDNA template (3).
- Resistant to amplification inhibitors present in template DNA isolated from problematic samples (4,5).

Assay Conditions:

40 mM Tris-HCl (pH 8.5 at 22°C), 1 mM $MnCl_2$, 1 mg/ml bovine serum albumin, 10 mM dithiothreitol, 0.5 mM [α-^{32}P] dTTP and 0.4 mM poly(A)·$(dT)_{50}$. Incubation is at 50°C for 10 min in a reaction volume of 50 µl.

Storage Buffer:

50 mM Tris-HCl (pH 7.5 at 22°C), 5 mM dithiothreitol, 0.1 mM EDTA, 50% (v/v) glycerol and stabilizers.

10 x Reaction Buffer:

670 mM Tris-HCl (pH 8.9 at 22°C), 166 mM ammonium sulfate, 0.1% Tween™20.

Quality Control:

All preparations are assayed for contaminating endonuclease, exonuclease, nonspecific RNase and single- and double-stranded DNase activities.

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Example Reaction:

RT

1. Mix:

Component	Final concentration/ Amount	Add per reaction
10 x Tth RT Buffer	1 x	2 µl
dNTP's mix (5 mM each)**	0.25 mM	1 µl*
Reverse Primer (10 µM)	20 pmol	2 µl*
Tth RT	0.25 U/µl	-
50 mM $MnCl_2$	2 mM	0.8 µl
Template RNA	5 ng-1 µg	-
Nuclease-free water	-	to 20 µl

2. Incubate 3 min at 53°C for primer annealing (primer dependent temperature) followed by 10-20 min at 60°C and add 80 µl of PCR mix:

PCR

Component	Final concentration/ Amount	Add per reaction
EGTA 50 mM	0.75 mM	1.2 µl
10 x Pol Buffer A	1 x	8 µl
dNTP's mix (5mM each)**	0.2 mM	3.2 µl*
Reverse Primer (10 µM)	80 pmol	8 µl*
Forward Primer (10 µM)	100 pmol	10 µl
25 mM $MgCl_2$	2 mM	6.4 µl
Nuclease-free water	-	to 80 µl

Notes:

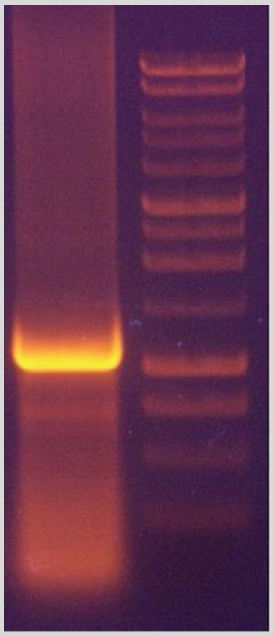
*dependent on concentration of stock solution

**e.g. dNTP Set (100 mM each), Cat. No. E0502

Total reaction volume 100 µl.

3. Run PCR program suitable for your primers and template. Since Tth Reverse Transcriptase has DNA polymerase activity there is no need to add additional DNA polymerase. Polymerization temperature for Tth DNA Polymerase is 72°C.

Keep in mind that processivity of Tth DNA Polymerase is quite low, up to 2 kb could be amplified but the most suitable are fragments below 1 kb. As compared to two-step RT procedures the error rate of the polymerase is elevated. This is due to the presence of manganese ions (1).



RT-PCR using Tth Reverse Transcriptase

M – Perfect Plus 1 kb DNA Ladder (Cat. No. E3131)

1 – 1137 nt fragment of *Sus scrofa* arginase transcribed and amplified with Tth Reverse Transcriptase from total RNA isolated from liver using GeneMATRIX Universal RNA Purification Kit (Cat. No. E3598).

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