EURx® MOLECULAR BIOLOGY PRODUCTS

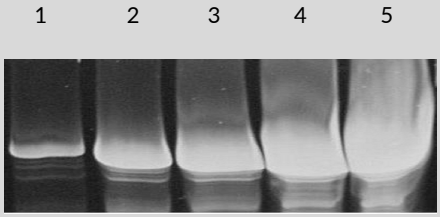
T7 RNA Polymerase

(bacteriophage T7 of Escherichia coli)

Cat. No.	size
E1290-01	5 000 units
E1290-02	25 000 units

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of labeled UTP into acid-insoluble material in 1 hr at 37°C.

Storage Conditions: Store at -20°C.



T7 RNA transcription of 400 nt RNA.
5 µl of T7 transcription were mixed with 5 µl of 2 x RNA loading buffer and loaded on 7% of polyacrylamide gel supplemented with 8 M urea. The gel was ethidium bromide stained. Lanes 1-5: respectively 50, 100, 200, 400, 800 U of T7 RNA Polymerase used for T7 transcription.

Modified T7 RNA Polymerase with higher tolerance towards modified nucleotides. Extremely useful for radioactive and non radioactive labeling as well as for RNA synthesis for preparative scale.

Description:

- DNA-dependent RNA polymerase which has stringent specificity for T7 phage promoters sequence (1).
- Ultrapure recombinant enzyme.
- Efficiently synthesizes *in vitro* transcripts from almost any DNA that is downstream from a T7 promoter (2).
- Suitable for preparing labeled single-stranded RNA probes of high specific activity (3).
- Transcripts can be used as hybridization probes, templates for *in vitro* translation, substrates in RNA processing systems, or exon and intron mapping of genomic DNA.

Storage Buffer:

20 mM potassium phosphate (pH 7.7), 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol and 50% (v/v) glycerol.

T7 in vitro transcription, example reaction protocol:

Component	Final Concentration/ Amount	Add per reaction
5 x T7 Reaction Buffer	1 x	10 µl
NTPs mix (25 mM each)	2 mM per NTP	4 µl*
Thermophilic Pyrophosphatase 5U/µl (optional; EURx Cat. No. E1267)	2.5 U	0.5 µl*
DNA template for T7 transcription	2 µg	Variable
T7 RNA Polymerase	50-800 U**	Variable
RNase-free water	-	Variable
Total volume	50 µl	-

*dependent on concentration of stock solution
**50 U is most efficient for labeling, more units are recommended for preparative scale

Incubate up to 2 hours at 37°C and check transcription on appropriate denaturing polyacrylamide gel. Load 5 µl of reaction mixed with 5 µl of 2 x RNA loading buffer (2.6 M urea, 2 x TBE, 0.02% (w/v) BPB, 0.02% (w/v) XCB, 66% (v/v) formamid).

Quality Control:

All preparations are assayed for contaminating exonuclease, endonuclease and nonspecific RNase and single- and double-stranded DNase activities. Typical preparations are greater than 90% pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. *Chamberlin, M. and Ring, J. (1973) J. Biol. Chem. 248, 2235-2244.*
2. *Tabor, S and Richardson, C.C. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 1074-1078.*
3. *Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, second edition, pp. 10.27-10.37, Cold Spring Harbour Laboratory, Cold Spring Harbour.*

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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