T7 transcription Kit

(bacteriophage T7 of Escherichia coli)

Cat. No.	size
E0901-01	25 x 25 µl reactions
E0901-02	50 x 25 µl reactions

T7 transcription Kit with modified T7 RNA Polymerase for higher tolerance towards modified nucleotides. Extremely useful for radioactive and nonradioactive labeling as well as for RNA synthesis in preparative scale.

Unit Definition:

One unit is 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.

Quality Control:

All preparations are assayed for contaminating exonuclease, endonuclease, for 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.*

Description:

- DNA-dependent RNA polymerase with stringent specificity for T7 phage promoters sequence (1).
- Ultrapure recombinant enzyme.
- Efficiently synthesizes *in vitro* transcripts from almost any DNA target located 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, as well as for 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.

Kit components:

COMPONENT:	E0901-01	E0901-02
5 x T7 Reaction Buffer	150 µl	300 µl
NTPs mix 25 mM each	50 µl	100 µl
T7 RNA Polymerase	12.5 µl	25 µl
RNase-free Water	1 ml	1 ml

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In vitro T7 transcription 25 µl:

Reaction assembly should be performed at room temperature (not on ice). This prevents any precipitation of template DNA due to spermidine contained in the 5 x T7 Reaction Buffer.

1. Mix:

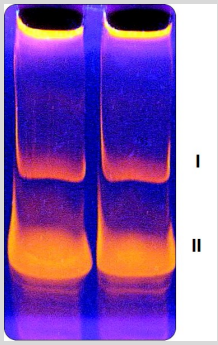
Component:	Amount:
5 x T7 Reaction Buffer	5 µl
NTPs mix 25 mM each	2 µl
DNA template*	1-2 µg
T7 RNA Polymerase**	0.5 µl
RNase-free Water	to 25 µl

2. Incubate up to 2 hours at 37°C, then check transcription on appropriate denaturing polyacrylamide gel.

NOTES:

*High purity of the template is very important for the yield of reaction. If run off transcription is applied be sure there is no RNase A contamination that could be due to plasmid preparation. We recommend using our RNase-free Plasmid DNA Purification kit (Cat. No. E3500), which works excellent for preparing RNase-free plasmid DNA. In case, T7 template DNA is a PCR fragment, remove primers (recommended: purification from agarose gels using e.g. our Agarose-Out DNA Purification Kit, Cat. No. E3540) and confirm DNA homogeneity on an agarose gel.

**0.2 µl of T7 RNA Polymerase is most efficient for labeling, more enzyme is recommended for preparative scale.



T7 transcription of 400 nt RNA using EURx T7 Transcription Kit. 3 µl of transcription was loaded on a 7% [w/v] polyacrylamide gel with 8 M urea.

I– DNA template.

II– 400 nt RNA product of T7 RNA Polymerase.

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