MANUFACTURED BY EURx

roboklon

T7 Transcription Kit

(Bacteriophage T7 of *Escherichia coli*)

T7 RNA Polymerase

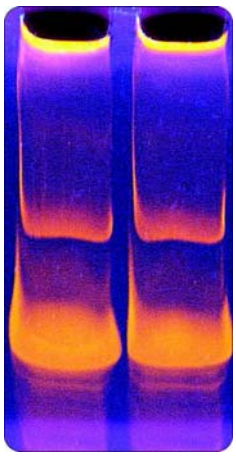
– modified, optimized –
(Bacteriophage T7 of E. coli)

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

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

Storage Conditions:
Store at -20°C



T7 transcription of 400 nt RNA using EURx T7 transcription kit. 3 µl of transcribed RNA was loaded on a 7 % (w/v) polyacrylamide gel with 8 M urea. Upper band, DNA template; lower band, 400 nt transcribed RNA product of T7 RNA polymerase.

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.

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.

Description:

- → T7 transcription is known to be extremely sensitive towards high quality and purity of all components. A single poor-quality component can cause reaction yield to drop in orders of magnitude. To guarantee optimum performance and maximum yield without compromise, this T7 transcription kit contains a complete set of all required, carefully adjusted reagents.
- → DNA-dependent RNA polymerase with stringent specificity for T7 phage promotor sequences (1).
- → Ultrapure recombinant enzyme.
- → Efficiently synthesizes *in vitro* transcripts from almost any DNA 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:

Kit component	25 Rxn.	50 Rxn.
5x reaction buffer	150 µl	300 µl
NTPs mix 25 mM each	50 µl	100 µl
DTT [100 mM]	50 µl	100 µl
Thermostable Pyrophosphatase 2.5 U/µl	12.5 µl	25 µl
T7 RNA Polymerase	12.5 µl	25 µl
RNase-free water	1 ml	1 ml
RNA loading buffer	50 µl	100 µl

T7 *in-vitro* Transcription Protocol:

Reaction assembly should be performed at room temperature (not on ice). This prevents any precipitation of template DNA due to spermidine-supplemented 5x reaction buffer.

5 µl	T7 RNA Polymerase 5 x reaction buffer
1.8 µl	NTPs mix, 25 mM each
1.25 µl	DTT 100 mM
0.5 µl	Thermostable Pyrophosphatase [2.5 U/µl] = 1.25 U
1-2 µg	DNA template*
0.5 µl	T7 RNA Polymerase**
@25 µl	RNase-free water

Total Reaction Volume 25 µl

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

Load 5 µl of reaction mixed with 3 µl of RNA loading buffer.

* High purity of template DNA is very important for obtaining high reaction yields. If run off transcription is applied, be sure there remains no RNase A contamination that could be due to insufficient plasmid preparation. Kits from various manufacturers show pronounced differences in their capabilities for completely removing RNases from plasmid DNA. We recommend using our RNase free Plasmid DNA Purification kit (Cat. No. E3500), which has been specifically tested for this application and works excellent for preparing RNase free plasmid DNA. In case, T7 template DNA is a PCR fragment, remove primers (Recommended procedure: Purification from Agarose Gels using e.g. our AgaroseOUT DNA Kit, Cat. No. E3540) and confirm DNA homogeneity on an agarose gel.

**0.2 µl of T7 RNA polymerase is efficient for labeling, higher amounts of enzyme may be required when working in preparative scale.