EURx® MOLECULAR BIOLOGY PRODUCTS

Thermo T3 Transcription Kit

(bacteriophage T3 of Escherichia coli)

Cat. No.	size
E0907-01	25 x 25 µl reactions
E0907-02	50 x 25 µl reactions

Thermo T3 Transcription Kit with modified T3 RNA Polymerase for higher efficiency up to 50°C. 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.Claire E. Morris et.al. and William T. McAllister, (1985) Gene 41(1986) 193-200.

Description:

- DNA-dependent RNA polymerase with stringent specificity for T3 phage promoters sequence (1) and increased thermostability. Activity range: 37-51°C.
- Ultrapure recombinant enzyme.
- Efficiently synthesizes *in vitro* transcripts from almost any DNA target located downstream from a T3 promoter.
- Suitable for preparing labeled single-stranded RNA probes of high specific activity.
- 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:	E0907-01	E0907-02
5 x T3 Reaction Buffer	150 µl	300 µl
NTPs mix, 25 mM each	50 µl	100 µl
Thermo T3 RNA Polymerase	12.5 µl	25 µl
RNase-free Water	1 ml	1 ml

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In vitro Thermo T3 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 T3 Reaction Buffer.

1. Mix:

Component:	Amount:
5 x T3 Reaction Buffer	5 µl
NTPs mix, 25 mM each	2 µl
DNA template*	1-2 µg
Thermo T3 RNA Polymerase**	0.5 µl
RNase-free Water	to 25 µl

2. Incubate up to 2 hours at 37-51°C (the best performance is observed at 46°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 EURx RNase-free Plasmid DNA Purification Kit (Cat. No. E3500), which works excellent for preparing RNase-free plasmid DNA. In case, T3 template DNA is a PCR fragment, remove primers (recommended: purification from agarose gels using e.g. EURx Agarose-Out DNA Purification Kit, Cat. No. E3540) and confirm DNA homogeneity on an agarose gel.

**0.2 µl of Thermo T3 RNA Polymerase is most efficient for labeling, more enzyme is recommended for preparative scale.

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