

EZ™ T7 High Yield In Vitro Transcription Kit

Cat.# **EZ0275** Size **75 reactions**

Expire date:

Store at -20°C

Supplied with: 5X Transcription Buffer
10X MgCl₂
rATP, rGTP, rCTP, rUTP(100 mM)
Control Template DNA (1 µg/µl)
T7 RNA Polymerase (200 U/µl)
DTT (100 mM)
20X Enhancer Solution
Sterile water (RNase free)

For Research Use Only. Not for use in diagnostic procedures.

ISO9001 ISO14001 ISO13485

※ If using this kit for the first time, it is highly recommended to visited our website and to read the detailed protocol.

Product description

T7 phage RNA polymerases are widely used for the in vitro synthesis of RNA transcripts from DNA templates. Enzynomics' EZ™ T7 High Yield In Vitro Transcription Kit is designed for high yield in vitro transcription from DNA templates containing T7 RNA Polymerase promoter. The phage RNA polymerases have a high specificity for their respective promoters. The complete kit includes the RNA polymerases, all of the required reagents for performing transcription reactions in vitro (excluding radioisotope). The kit contains sufficient reagents for 75 reactions of 20 µl each. Each standard reaction yields up to 180 µg of RNA from 1 µg control template in 1 hour. The template must have a double-stranded 19-23 base promoter upstream of the sequence to be transcribed. The kit can be successfully used to produce both long and short RNA transcripts. The RNA synthesized is suitable for variety of applications that require large amounts of RNA, such as in vitro translation, antisense RNA and RNAi studies, RNase protection assays, studies of RNA splicing, isolation of RNA binding proteins. Non-radioactively labeled RNA can be used as probes in microarrays, blots or in situ hybridization.

Standard reaction condition

1) Assemble the following reaction components at room temperature in the order listed.

5X Transcription Buffer	4 µl
10X MgCl ₂	2 µl
DTT (100 mM)	2 µl
20X Enhancer Solution	1 µl
rATP (100 mM)	1 µl
rGTP (100 mM)	1 µl
rCTP (100 mM)	1 µl
rUTP (100 mM)	1 µl
Template DNA	x µl
RNA polymerase	1 µl
Sterile water	up to 20 µl

* To increase the yield of RNA, add 10 mM of rNTP and 1X MgCl₂ in a standard transcription reaction.

2) Mix thoroughly, spin briefly to collect all drops and incubate for 1 hour at 37°C

- 3) Add DNase I to a concentration of 1 unit/µg of template DNA
- 4) Incubate for 30 minutes at 37°C
- 5) Add 5 µl from the transcription product to 15 µl RNA sample buffer.
- 6) Add 2-5 µl of RNA loading buffer and heat the samples for 10 minute at 60°C prior to loading on to agarose gel.

Additional required materials which not provided

- DNase I
- RNA Ladder Marker
- RNA Loading dye / RNA Loading buffer
- RNA Sample buffer

Note

- The mixture should be kept at room temperature while each successive component is added, since DNA can precipitate in the presence of spermidine if kept at 4°C.
- Avoiding RNase Contamination
- RNA frozen in Transcription buffer, as it will precipitate at low temperatures in the presence of spermidine. RNA stored in this way will not run to its true size upon electrophoresis.
- If the DNA template is not removed, a high-molecular-weight band (~4kb for the control template DNA) will be visible.
- Enhancer Solution is consist of RNase Inhibitor and enzyme cocktail for the stimulation of RNA synthesis

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