T7 RNA polymerase

Catalogue number:

MB08001, 10000 U (20 U/µL)
MB08003, 10000 U (200 U/µL)

Description

T7 RNA polymerase is a recombinant enzyme purified from *Escherichia coli*, for the synthesis of high specific-activity RNA probes, biologically active mRNA and antisense RNA. T7 RNA polymerase is DNA-dependent with strict specificity for its own double-stranded promoter that is not efficiently recognized by SP6 or T3 RNA polymerases. T7 RNA polymerase catalyzes the 5'→3' synthesis of RNA from ribonucleoside triphosphates on single or double-stranded DNA downstream from a T7 promoter. Using circular plasmid DNA as a template will result in heterogeneous transcripts of multiple lengths. T7 RNA polymerase accepts modified nucleotides as substrates for RNA synthesis.

Storage temperature

T7 RNA polymerase should be stored at -20 °C in a constant temperature freezer. The enzyme is stable up to the expiry date specified with the product.

Unit definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of rATP into acid insoluble material in 60 minutes at 37 °C, under the following assay conditions: 40 mM Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 10 mM DTT, 2 mM spermidine, 0.5 mM each of rATP, rCTP, rGTP, rUTP, 0.6 MBq/ml [^{3}H]-rATP, 20 µg/ml of DNA containing the specific T7 RNA polymerase promoter sequence in a final volume of 50 µL.

Enzyme concentration

20 U/µL (cat. No. MB08001) or 200 U/µL (cat. No. MB08003).

Reaction buffer (10×)

400 mM Tris-HCl, pH 7.9, 60 mM $MgCl_2$, 100 mM DTT, 20 mM Spermidine. Vortex the 10× Reaction buffer solution thoroughly after thawing and prior to use. Repeated freeze-thaw cycles will affect the stability of the buffer (it remains stable at 4 °C up to one month).

Enzyme Inactivation

T7 RNA polymerase is heat inactivated at 70 °C for 10 min.

Protocol for a typical RNA synthesis reaction

The transcription reaction should be performed under appropriate conditions that exclude contamination with RNases.

1. In a sterile nuclease-free microcentrifuge tube, **at room temperature**, prepare a reaction mixture containing the following components (the mixture can be scaled up or down):

Component	Volume
10× Reaction buffer (provided)	2 µL
rNTP mix, 25 mM solution	0.5-1.0 µL
Linearized template DNA	1 µg
T7 RNA polymerase	20 U
RNase Inhibitor* (final concentration)	0.1-1.0 U/µL
Nuclease-free water	up to 20 µL

*We recommend using NZY Ribonuclease Inhibitor (Cat. No. MB084)

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.

3. Incubate at 37 °C for 2 hours.

4. If necessary, treat the reaction with DNase I to remove DNA template (see step 5) or, in alternative, stop the transcription reaction by adding 2 µL EDTA at 0.2 M and/or heating at 70 °C for 10 minutes.

5. (Optional) *Procedure for removing DNA template:*
Add 2 U DNase I (not provided) directly to the transcription mixture and incubate at 37 °C for 15 minutes. Then, inactivate the DNase I by adding 2 µL EDTA at 0.2 M followed by heating at 70 °C for 10 minutes, or by phenol/chloroform extraction.

6. After the transcription reaction, proceed with purification of synthesised RNA and/or analysis of integrity, length and yield of the transcript on agarose gel.

Quality control assays

Purity

Recombinant T7 RNA polymerase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe (Cat. No. MB15201) staining.

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 20 U of T7 RNA polymerase for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 20 U of T7 RNA polymerase for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. Similar tests are performed with the reaction buffer.

Functional assay

T7 RNA polymerase and the respective reaction buffer are functionally tested in an *in vitro* transcription reaction using a linearized recombinant plasmid (pET28-derivative containing a 600 bp DNA fragment). The result must be a 600 bp band correspondent of the desired RNA transcript observed on a GreenSafe-stained agarose gel.

Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nucleases assays	Pass
Functional assay	Pass

Approved by:

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