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genes & enzymes

T7 RNA polymerase

Catalogue number: MB08001, 10,000 U (20 U/µL)
MB08002, 30,000 U (20 U/µL)
MB08003, 10,000 U (200 U/µL)
MB08004, 30,000 U (200 U/µL)

Description

T7 RNA polymerase is a recombinant enzyme purified from *Escherichia coli*, for the synthesis of highly 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.

Storage buffer

50 mM Tris-HCl, pH 7.9, 150 mM NaCl, 10 mM DTT, 1 mM EDTA, and 50% (v/v) glycerol.

Unit definition

One unit is defined as the amount of enzyme required to catalyse 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 (catalogue #MB08001 and #MB08002) or 200 U/µL (catalogue #MB08003 and #MB08004).

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 (the buffer will remain stable at 4 °C up to one month).

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 conditions that exclude contamination with RNases. 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

*NZY Ribonuclease Inhibitor (catalogue #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. Treat the reaction with DNase I to remove DNA template (see 5.) or stop the transcription reaction by adding 2 µL 0.2 M EDTA and/or heating at 70 °C for 10 minutes.

5. (Optional) Remove DNA template: digest with 2 U DNase I at 37 °C for 15 minutes. Inactivate the DNase I by adding 2 µL 0.2 M EDTA and heating at 70 °C for 10 minutes, or by phenol/chloroform extraction.

Quality control assays

Purity

Recombinant T7 RNA Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining.

Nuclease assays

To test for DNase activity, 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 activity, 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.

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Certificate of Analysis

Test	Result
Enzyme purity	Pass
Nuclease assays	Pass

Approved by:

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