T7 RNA polymerase

Catalogue number	Presentation
MB08001	10000 U (20 U/µL)
MB08003	10000 U (200 U/µL)

Description

T7 RNA Polymerase is a highly purified recombinant enzyme produced in *Escherichia coli*. It is widely used for the *in vitro* synthesis of high-specific-activity RNA probes, biologically active mRNA, and antisense RNA. This enzyme plays a critical role in applications such as transcription assays, RNA labeling, and the production of synthetic RNAs for functional and structural studies. T7 RNA Polymerase is a DNA-dependent RNA polymerase with stringent specificity for its own double-stranded T7 promoter, which is not recognized by SP6 or T3 RNA polymerases, ensuring high specificity in transcription. The enzyme catalyzes RNA synthesis in the 5'→3' direction, utilizing ribonucleoside triphosphates (rNTPs) as substrates. Transcription occurs on single- or double-stranded DNA templates positioned downstream of the T7 promoter. When circular plasmid DNA is used as a template, the transcription process may produce heterogeneous RNA transcripts of varying lengths due to multiple transcription initiation events. T7 RNA Polymerase can also incorporate modified nucleotides, expanding its utility for diverse labeling and detection applications.

Shipping & Storage Conditions

The product can be shipped from dry ice to blue ice. Upon receipt, store all components at -30 °C to -15 °C in a constant temperature freezer. Stability can be extended by storing them at -85 °C to -65 °C. Repeated freeze-thaw cycles can compromise the stability of the reaction buffer provided. To minimize the impact, aliquot it into small volumes after the first thawing. Adhering to these meticulous storage procedures ensures that product delivers consistent and reliable results across its lifespan and usage. Stored as specified, the product will remain stable until the expiry date, ensuring reliable and consistent performance in all applications.

Components

	MB08001 (10000 U, 20 U/µL)		**MB08003 (10000 U, 200 U/µL)**	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
T7 RNA polymerase (20 U/µL)	1	500 µL	-	-
T7 RNA polymerase (200 U/µL)	-	-	1	50 µL
10x Reaction Buffer for T7 RNA polymerase	1	1 mL	1	1 mL

Specifications

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 MgCl2, 10 mM DTT, 2 mM spermidine, 0.5 mM each of rATP, rCTP, rGTP, rUTP, 0.6 MBq/ml [3H]-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).

Inhibition & Inactivation: T7 RNA polymerase is inhibited in the presence of metal chelators (e.g. EDTA) and high salt concentrations (NaCl or KCl at concentration > 150-200 mM). The enzyme is inactivated at 70 °C for 10 min.

Standard Protocol

Recommendations before starting

- Reagent usage:
  - The reaction buffer provided contains DTT. Before use, thoroughly vortex the buffer solution after thawing. Repeated freeze-thaw cycles will affect the stability of the buffer – refer to section Shipping & Storage above.

- The reaction buffer provided already contains Mg2+ at the optimal concentration for T7 RNA polymerase activity. In extremely rare cases, the addition of extra Mg2+ for concentration adjustments may be considered.
- It is strongly advised to include a ribonuclease inhibitor in the reaction. Incorporating a ribonuclease inhibitor into the reaction further safeguards synthesized RNA from degradation, ensuring the reliability and accuracy of downstream analyses. Please consult the NZYtech portfolio for available Ribonuclease Inhibitors.
- We highly recommend using sterile molecular biology-grade, nuclease-free water, preferably DEPC-treated water

▪ Handling instructions:
- During setup, place the enzyme on ice but keep the 10× Transcription Reaction Buffer at room temperature. Some components present in the Reaction Buffer can co-precipitate the template DNA if the reaction is assembled on ice.
- The reaction setup should be executed under stringent conditions to prevent contamination with RNases. This includes working in a dedicated RNase-free environment, using RNase-free reagents and consumables, and wearing gloves to minimize the introduction of RNases from the skin. Additionally, it is crucial to regularly clean and decontaminate work surfaces and equipment. The RNase & DNase Cleaner (NZYtech, Cat. No. MB463) can help removing RNases from surfaces and materials.

Procedure for *in vitro* transcription

1. Gently mix the enzyme component and pulse briefly. Vortex the Reaction buffer thoroughly.
2. **At room temperature**, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture by combining the following components in the order indicated:
   Note: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed.*

COMPONENT	1 REACTION VOLUME
Nuclease-free water (not provided)	up to 20 µL
10× Reaction Buffer (provided)	2 µL
rNTP mix, 25 mM solution	0.5-1 µL
Linearized template DNA	0.5-1 µg
Ribonuclease Inhibitor (not provided *)	0.1-1.0 U/µL
T7 RNA polymerase	20 U
FINAL VOLUME =	20 µL

(*) Review the recommendations for reagent usage before starting (section above).

3. Mix thoroughly and centrifuge briefly to collect the contents at the bottom of the tube.
4. Incubate at 37 °C for 2 hours.
   Note: *For short transcripts (<0.3 Kb), incubate the transcription reaction at 37 °C for 4 hours or more.*
5. **(Optional)** To remove the DNA template, treat the reaction with DNase I. To achieve this, add 2 U DNase I (not provided, but ensure that it is RNase-free) directly to the transcription mixture and incubate at 37 °C for 15 minutes. Then, inactivate the DNase I and the T7 RNA polymerase by adding 2 µL EDTA at 0.2 M followed by heating at 70 °C for 10 minutes, or by phenol/chloroform extraction.
6. If not performing the DNase I treatment, inactivate the reaction by adding 2 µL EDTA at 0.2 M and/or heating at 70 °C for 10 minutes.
7. Store RNA produced at - 85 °C to -15 °C or proceed to RNA analysis and/or downstream applications.

Technical Notes

DNA Template Type
Any linearized plasmid or PCR products containing a T7 promoter region may be used as a template for *in vitro* transcription using the T7 RNA polymerase.

Plasmid templates: For successful *in vitro* transcription, plasmid templates must be of high quality and linearized. Linearization ensures the production of RNA transcripts with defined lengths, as circular plasmids result in long, heterogeneous transcripts due to the processive nature of T7 RNA polymerase. To generate RNAs of precise lengths, digest the plasmid DNA at the 3' region where transcription should stop, preferably using restriction enzymes that create blunt ends or 5' overhangs. If 3' overhangs are generated, treat the ends with the Klenow Fragment of DNA polymerase I (NZYtech, Cat. No. MB009) to create blunt ends before transcription.

PCR templates: DNA templates generated by PCR and containing a T7 promoter can be efficiently transcribed using the NZY T7 RNA Synthesis Kit. Verify their concentration and size on an agarose gel and purify the products to ensure optimal transcription yields.

Quality control

Purity

T7 RNA polymerase is >90% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 DNA are incubated with 20 U of T7 RNA polymerase for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 20 U of the enzyme for 1 h 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 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.

NZYtech Lda. Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.: +351 213643514
Fax: +351 217151168 www.nzytech.com