Data Sheet

Jena Bioscience
Building Blocks of Life

T7 RNA Polymerase

RNA Polymerase

***in vitro* Transcription**

	Cat. No.	Size	Conc.
	PCR-603S	4,000 units	200 units/µl
	PCR-603L	5 x 4,000 units	200 units/µl

For general laboratory use

Shelf Life: Quality guaranteed for 12 months

Storage Conditions: Store at -20°C, avoid frequent thawing and freezing

T7 RNA Polymerase (red cap)
200 units/µl in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM DTT, 0.1 mg/mL BSA, 0.03% (v/v) ELUGENT detergent, 50% (v/v) glycerol

T7 Reaction Buffer (green cap)
5 x conc.
200 mM Tris-HCl (pH 7.9), 30 mM $MgCl_2$, 50 mM DTT, 50 mM NaCl, 10 mM spermidine

Description
T7 RNA polymerase is a DNA-dependent RNA polymerase that catalyzes the synthesis of RNA starting from a T7 promoter sequence. The enzyme guarantees efficient transcription of DNA templates containing a T7 promoter site.

Applications
- In vitro RNA synthesis
- RNA probe synthesis
- Generation of RNA template for *in vitro* translation
- RNAi (RNA interference) study

Unit Definition
One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction in 60 min at 37°C.
Enzyme activity is assayed in the following mixture: 40 mM Tris-HCl (pH 8.0), 6 mM $MgCl_2$, 10 mM DTT, 2 mM spermidine, 0.5 mM each NTP, [3H]-ATP, 20 µg/ml plasmid DNA containing the appropriate promoter sequences.

Inactivation
Inactivated by incubation at 70°C for 10 min or by addition of EDTA.

Template DNA
In general, any DNA (linearized plasmid DNA, PCR products) containing a T7 promoter site can be used as template for T7 RNA polymerase.

Minimum promoter sequence for efficient transcription:

5'-TAATACGACTCACTATA**GGGAGA**...-3'

↳ Start of transcription

Recommended protocol

1. Assay preparation
Add the following components to a nuclease-free microtube and vortex gently:

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Component	stock conc.	final conc.	20 µl assay	50 µl assay
T7 Reaction Buffer	5x	1x	4 µl	10 µl
NTP Mix	10 mM each	500 µM	1 µl	2.5 µl
RNase Inhibitor [1]	40 units/µl	1 unit/µl	0.5 µl	1.25 µl
Template DNA with T7 promoter [2]			10-500 ng	10-1000 ng
T7 RNA Polymerase	200 units/µl	2 units/µl	0.2 µl	0.5 µl
nuclease-free water			fill up to 20 µl	fill up to 50 µl

1) Addition of 10-20 units RNase inhibitor per 20 µl assay is recommended (and may be essential when working with low amounts of template DNA)
2) For lower amounts of template DNA (<100 ng) an increased incubation time of up to 60 min is recommended.

2. Incubation
Incubate the mixture at 37°C for 30 min.

Please note: Incubation at 42°C may increase the yield by about 10%. For lower amounts of template DNA (<100 ng) an increased incubation time of up to 60 min is recommended.

3. Optional DNase treatment
Add 1 µl (1 MBU) RNase-free DNase to the reaction product and incubate for 15 min at 37°C to remove template DNA.