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Manual

T7 RNA polymerase

Recombinant T7 phage RNA polymerase. Concentration 20 U/µl.

catalog #	size
1014-5	5000 U

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Description

T7 RNA polymerase is a recombinant enzyme produced by *E.coli* strain carrying a DNA plasmid with a cloned gene of T7 phage RNA polymerase.
It is DNA-dependent RNA polymerase. Enzyme catalyzes 5'-3'RNA synthesis on ssDNA or dsDNA. T7 RNA polymerase is very specific for the bacteriophage T7 promoter - transcription starts from T7 promoter only.

Application

T7 RNA polymerase is suitable for labeled and unlabeled RNA synthesis:

- as hybridization probes
- for *in vitro* RNA translation
- for genome mapping
- for *in vitro* transcription and high-yield *in vitro* transcription

Contents

	1014-5	**storage**
T7 RNA polymerase	5000 U (20 U/µl)	-20 °C
T7 buffer 5x transcription buffer: 1M HEPES-KOH, pH 7.6, 150 mM $Mg(CH_3COO)_2$, 200 mM DTT	1 x 1.25 ml	-20 °C

Additional equipment and reagents

Necessary

- NTPs mix

Optional

- RNAse inhibitor (cat. # 037-25, 037-100, 037-1000)
- ultrapure water

Example *in vitro* RNA transcription protocol

1. Thaw all components on ice, gently mix by inverting the tubes and briefly centrifuge.
2. Add:

	PCR reaction volume
component	**20 µl**
linearized and purified DNA	50-200 ng
NTP mix	2 mM of each of NTPs
T7 buffer	4 µl
T7 RNA polymerase	10-20 U
RNAse inhibitor (optional)	10-20 U
ultrapure water	up to 20 µl

3. Reaction conditions: 1-2 hours at temp. 37 °C

Notes

- Optional, to remove traces of DNA, add 1-2 U of DNAse (cat. # 1009-10). Incubate for 15 min at 25-37 °C. Next, to inactivate DNAse, we recommend cleaning the RNA with the Clean-Up RNA Concentrator kit (cat. # 039-25C, 039-100C).
- T7 RNA polymerase can be used to perform high-yield *in vitro* transcription. Add more quantity of NTP mix (5 mM of each of NTPs) and pyrophosphatase (0.1-0.3 U).
- Yield of transcripts with specified length is reduced using incomplete linearized DNA.
- To limit contamination of RNAses, use sterile, RNAse-free disposable, diagnostics gloves, clean the laboratory surfaces with a suitable agent, e.g. LabZAP (cat. # 040-500).

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