T7 RNA Polymerase

T7 RNA Polymerase catalyzes the formation of RNA from a DNA template in the 5'-3' direction and is commonly used for in vitro (IVT) applications.

The enzyme is T7 promoter specific, requires Mg^{2+} as a cofactor and can use modified nucleotides for the synthesis. The T7 RNA Polymerase requires a double-stranded DNA template and can produce full-length RNA transcripts.

***in vitro* transcription of RNA**

Key Features

- T7 promoter-specific RNA polymerase
- Available in glycerol-based formulation and lyophilization-friendly formulation without glycerol

Suggested Applications

- *in vitro* transcription of RNA
- Molecular diagnostics (NASBA and other)

Lyophilization friendly

Properties

Source	Recombinantly produced in *E. coli*.
Size	99.97 kDa
Inactivation	NA
Storage and stability	The enzyme is stable at -20°C for up to 1 year in the supplied buffer. It is recommended to avoid repeated freeze-thaw of formulations not containing glycerol.

Suggested IVT Protocol

Component	Volume	Final
5X Reaction buffer	10 µl	1X
ATP/GTP/CTP/UTP (10 mM)	10 µl	2 mM each
Linear template DNA	variable	1 µg
RNase Inhibitor (optional)	variable	1 U/µl
T7 DNA Polymerase (50 U/µl)	0.6 µl	0.6 U/µl
DEPC-treated water	to 50 µl	-

1. At room temperature, prepare a reaction mix as described in Suggested IVT protocol.
2. Incubate the reaction mix at 37°C for 1-2 hours. For highest RNA yield, incubate for 2 hours. Stop the reaction by adding 10 µl 60 mM EDTA.

Suggested 5X reaction buffer: 200 mM Tris-HCl pH 8.0 (25°C), 10 mM Spermidine, 50 mM DTT and 30 mM $MgCl_2$.
Note: Reaction conditions may vary with application and require optimization

Quality Control

dsDNA endonuclease activity	100 U T7 RNA Polymerase was incubated with a supercoiled plasmid (1 µg) for 4 hours at 37°C. Agarose gel electrophoresis did not reveal any transformation of closed circular DNA to nicked DNA.
ssDNA endonuclease activity	200 U T7 RNA Polymerase was incubated with M13 ssDNA (0.5 µg) for 4 hours at 37°C. Agarose gel electrophoresis did not reveal any visible signs of ssDNA degradation.
ssDNA and dsDNA exonuclease activity	200 U T7 RNA Polymerase was incubated with 3H-dATP labelled ds or ssDNA (0.5 µg, 500 bp) for 4 hours at 37°C. Acid soluble radioactivity from labelled DNA was not significant over blank test for either substrate.
RNase activity	200 U T7 RNA Polymerase was incubated with a 2 kb RNA transcript (1 µg) for 4 hours at 37°C. Agarose gel electrophoresis did not reveal any visible signs of RNA degradation.
Protein Purity	Protein purity of T7 RNA Polymerase was ≥95% as determined by SDS-PAGE analysis.

Ordering Information

	Article no.	Pack Size	Concentration
T7 RNA Polymerase	74100-201	10000 U	50 U/µl
	74100-110	100 kU	≥ 500 U/µl
	74100-100	As per request	As per request
T7 RNA Polymerase Glycerol Free	74110-110	100 kU	≥ 500 U/µl
	74110-100	As per request	As per request

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Quality

ArcticZymes is dedicated to the quality of its products and is certified according to ISO 13485. ArcticZymes offers the convenience of providing standard bulk enzymes as off the shelf products. In addition, ArcticZymes offers enzymes in customized formats. For additional information, please contact us.

Contact Information

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Additional Information

We are pleased to provide additional data and information relating to T7 RNA Polymerase on request. For more information about our enzymes and services, please visit our website www.arcticzymes.com.

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