Recombinant *E. coli* T7 phage RNA polymerase His-tag

Catalog Number: 11662-T7

DESCRIPTION

Source	*E. coli*-derived *e. coli* T7 phage RNA polymerase protein Asn2-Ala883 with a N-terminal Met and 6-His tag Accession # P00573.2
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	100 kDa

SPECIFICATIONS

SDS-PAGE	89-98 kDa, under reducing conditions
Activity	Measured by its ability to produce 4.1 kB RNA per agarose gel electrophoresis from a defined linearized DNA plasmid template quantified using a fluorescent detection assay. The RNA yield is >2500 ng, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Sodium Phosphate, NaCl, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 20 mM Sodium Phosphate, 100 mM NaCl, 1 mM TCEP, and 10% (v/v) Glycerol, pH 7.5
- rT7 phage RNA polymerase His-tag (rT7 RNAP) (Catalog # 11662-T7)
- Template DNA: Linearized DNA Plasmid
- Ribonucleotides (ATP, CTP, GTP, UTP)
- Ultrapure DNase/RNase-Free Distilled Water
- DNase I
- RNA Fluorescent detection system
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rT7 RNAP to 60 ng/µL using Assay Buffer.
2. Dilute Template DNA to 20 ng/µL using Assay Buffer.
3. Create a ribonucleotide mix containing 20 mM of each using Assay Buffer.
4. Add 10 µL ribonucleotide mix, 5 µL rT7 RNAP, and 5 µL of Template DNA to an RNase-free tube. Create a negative control with Assay Buffer in place of rT7 RNAP.
5. Incubate reaction at 37 °C for 2 hours.
6. Add 30 µL Ultrapure DNase/RNase-Free Distilled Water to all reaction tubes.
7. Add DNase I to reaction tubes and incubate at 37 °C for 15 minutes to remove template DNA.
8. Precipitate and isolate RNA.
9. Resuspend RNA in Ultrapure DNase/RNase-Free Distilled Water.
10. Quantify RNA concentration with fluorescent detection kit.
11. Calculate RNA yield based on detection system.

Final Assay Conditions

Per Reaction:

- rT7 RNAP: 0.3 µg
- Template DNA: 0.1 µg
- Ribonucleotides: 10 mM each

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

Recombinant *E. coli* T7 phage RNA polymerase His-tag

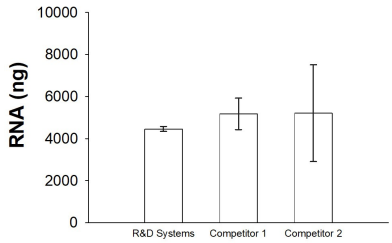
Catalog Number: 11662-T7

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

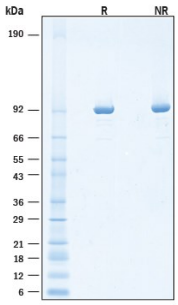
DATA

Enzyme Activity



Recombinant E. coli T7 phage RNA polymerase His-tag Protein Enzyme Activity. Recombinant T7phage RNA polymerase (T7 RNAP) (Catalog # 11662-T7) from R&D Systems and other competitors produce similar amounts of RNA in direct side-by-side comparison using the insert assay protocol described.

SDS-PAGE



Recombinant E. coli T7 phage RNA polymerase His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant E. coli T7 phage RNA polymerase His-tag Protein (Catalog # 11662-T7) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 89-98 kDa, under reducing conditions.

BACKGROUND

Recombinant T7 phage RNA polymerase (T7 RNAP) is a DNA-dependent RNA polymerase essential for T7 phage growth that is involved in the transcription of class II and class III viral DNA (1,2). T7 RNAP is a 99 kDa protein that consists of four accessory domains including an N-terminal single strand RNA binding domain, a four-helix bundle, the promoter recognition loop, and the C-terminal T7 lysozyme regulation domain in addition to the catalytic polymerase domain comprised of three sub-domains that form a deep cleft where the active site is located (3-5). The three catalytic polymerase domain sub-domains and active site residues are conserved throughout nucleic acid families (3). Unlike other polymerases from prokaryotic and eukaryotic sources, T7 RNAP is a single subunit enzyme and also requires no additional protein factors to complete a transcriptional cycle making it efficient and fast (6-9). T7 RNAP also has high specificity to the T7 promoter (9,10) and an ability to produce long transcripts (5). In addition, T7 RNAP has been shown to be amenable to structural manipulation for improved functionality or broader application (5). These characteristics confer an advantage to T7 RNAP to make it a broadly useful and effective tool for expression of genes under control of the T7 promoter in a variety of hosts for both in vivo and in vitro experiments (5,9). T7 RNAP is a useful tool in synthetic biology with many applications including synthesizing mRNA for vaccines and therapeutics, RNA editing and interference, expression control in biotech applications and industrial biotechnology, and as a model for transcription (9-11).

References:

1. Chung, Y.B. and D.C. Hickle (1990) J. Biol. Chem. **216**:927.
2. Zhang, X. and F.W. Studier (2004) J. Mol Biol. **340**:707.
3. Jeruzalmi, D. and T.A. Steitz (1998) EMBO J. **17**:4101.
4. Sousa, R. and S. Mukherjee (2003) Prog. Nucleic Acid Res. Mol. Biol. **73**:1.
5. Wang, W. *et al.* (2018) Biotechnol. Adv. **36**:2129.
6. Lyakhoc, D.L. *et al.* (1998) J. Mol. Biol. **280**:201.
7. Cheetham, G.M. and T.A. Steitz (2000) Curr. Opin. Struct. Biol. **10**:117.
8. Yunitskaya, V.L. and S.N. Kochetkov (2002) Biochemistry **67**:1124.
9. Borkotoky, S. and A. Murali (2018) Int. J. Biol. Macromol. **118**:49.
10. Rong, M. *et. al.* (1998) Proc. Natl. Acad. Sci. USA. **95**:515.
11. Nair, A. and Z. Kis (2024) Front. Mol. Biosci. **11**:1504876.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475