

CERTIFICATE OF ANALYSIS

T3 RNA Polymerase

#EP0102 10000u

Lot: **Quality guaranteed:**

Concentration: 20u/μl

Supplied with: 2x1.25ml of 5X Transcription Buffer

Store at -20°C

2

BSA included:
Lot# BSA62-313P

In total 3 vials.

Description

T3 RNA Polymerase is DNA-dependent RNA polymerase with strict specificity for its own double-stranded promoter. It catalyzes the 5'→3' synthesis of RNA from ribonucleoside triphosphates on single or double-stranded DNA downstream from a T3 promoter.

Source

E.coli cells carrying a cloned gene encoding this enzyme.

Unit Definition

One unit of the enzyme incorporates 1 nanomole of AMP into a polynucleotide fraction (adsorbed on DE-81) in 60min at 37°C.

Activity Assay

40mM Tris-HCl (pH 8.0), 6mM MgCl₂, 10mM DTT, 2mM spermidine, 0.5mM of each NTP, 0.6MBq/ml [³H]-ATP, 20μg/ml plasmid DNA containing the specific T3 RNA Polymerase promoter sequence.

Storage Buffer

50mM Tris-HCl (pH 8.0), 150mM NaCl, 5mM DTT, 0.1mg/ml BSA, 0.5mM ELUGENT Detergent and 50% glycerol.

5X Transcription Buffer

200mM Tris-HCl (pH 7.9 at 25°C), 30mM MgCl₂, 50mM DTT, 50mM NaCl and 10mM spermidine.

Applications

Generation of RNA sequences (see protocols on back page) that may be used as:

- probes for hybridization (1),
- genomic DNA sequencing (2),
- in RNase protection assays (3),
- antisense RNAs (4),
- templates for *in vitro* RNA translation (5),
- substrates for studies of RNA splicing (6), RNA secondary structure and RNA-protein interactions (7),
- templates for nucleic acid amplification,
- siRNA (8)

Inactivation

By heating at 70°C for 10min or the addition of EDTA.

Note

T3 RNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescein-labeled nucleotides) as substrates for RNA synthesis.

DE-81 – Whatman anion exchange chromatography paper having diethylaminoethyl functional groups.

Whatman is a registered trademark of Whatman Ltd.

ELUGENT is a trademark of Calbiochem Novabiochem, Inc.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 200 units of T3 RNA Polymerase with 1µg of pBR322 DNA in 50µl of buffer (40mM Tris-HCl (pH 8.0), 6mM MgCl₂, 10mM DTT) for 1 hour at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of T3 RNA Polymerase with 1µg of sonicated *E. coli* [³H]-DNA in 50µl of buffer (40mM Tris-HCl (pH 8.0), 6mM MgCl₂, 10mM DTT) for 1 hour at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 200 units of T3 RNA Polymerase with 1µg of [³H]-RNA in 50µl of buffer (40mM Tris-HCl (pH 8.0), 6mM MgCl₂, 10mM DTT) for 1 hour at 37°C.

Functional Assay

T3 RNA Polymerase was tested for use in the synthesis of strand-specific RNA sequences.

Quality authorized by:



Jurgita Zilinskiene

(continued on back page)

Protocol for synthesis of RNA

- 1 Linearize template DNA with a restriction endonuclease. Extract DNA with phenol /chloroform, and then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in water.
- 2 Prepare the following reaction mixture:

5X transcription buffer	10 μ l
10mM 4 NTP mix	10 μ l (2.0mM – final conc.)
linearized template DNA	1 μ g
Ribonuclease Inhibitor	50u
T3 RNA Polymerase	30u
DEPC-treated water	to 50 μ l
- 3 Incubate at 37°C for 60-120 minutes.
- 4 Stop the reaction by adding of 2 μ l 0.5M EDTA (pH 8.0) or by cooling at -20°C.
- 5 Analyze transcripts by electrophoresis.

Note

- The transcription reaction should be performed under conditions that exclude contamination with RNases. The tips, tubes and water should be nuclease free. All the solutions should be made up in nuclease free water. Wearing gloves is advisable.
- The reaction mixture should be prepared at room temperature, since DNA may precipitate in the presence of spermidine at 4°C.
- Under the conditions described above, more than 10 μ g RNA per 1 μ g template DNA is obtained.
- The yield of proper length transcripts decreases if the template DNA is incompletely linearized due to a read-through reaction and accumulation of longer transcripts of a variable length.
- The reaction mixture can be scaled up or down.

Protocol for Synthesis of High Specific Activity Radiolabeled RNA

- 1 Linearize template DNA with a restriction endonuclease. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in water.
- 2 Prepare the following reaction mixture:

5X transcription buffer	4 μ l
10mM 3 NTP mix	1 μ l (0.5mM – final conc.)
100 μ M CTP	2.4 μ l (12 μ M – final conc.)
[α - ³² P]-CTP, ~30TBq/mmol (800Ci/mmol)	1.85MBq (50 μ Ci)
linearized template DNA	0.2-1.0 μ g
Ribonuclease Inhibitor	20u
T3 RNA Polymerase	20u
DEPC-treated water	to 20 μ l
- 3 Incubate at 37°C for 60-120 minutes.
- 4 Stop the reaction by cooling at -20°C.
- 5 Determine the percentage of label incorporated.

Note

- RNA synthesized under the conditions described above usually has a specific activity of 3-5 x10⁸dpm/ μ g.
- RNA can be radiolabeled with [³²P], [³⁵S] or [³H]-ribonucleotides. The use of 1.85MBq (50 μ Ci) of 5'-[α -³²P]-CTP, ~30TBq/mmol (800Ci/mmol), 11.1MBq (300 μ Ci) of 5'-[α -³⁵S]-UTP, >37TBq/mmol (>1000Ci/mmol), 0.925MBq (25 μ Ci) of 5,6-[³H]-UTP, 1.1-2.2TBq/mmol (30-60Ci/mmol) for 20 μ l reaction mixture is recommended.

The yield of full-length transcripts is reduced when the final concentration of labeled NTP is below 12 μ M.

References

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5. Krieg, P.A., Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs, *Nucleic Acids Res.*, 12, 7057-7070, 1984.
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9. Jorgensen, E.D., et al., Specific contacts between the bacteriophage T3, T7, and SP6 RNA polymerases and their promoters, *J. Biol. Chem.*, 266, 645-651, 1991.

Related Products

- RiboLock™ Ribonuclease Inhibitor #E00381, #E00382
- Ribonuclease Inhibitor #E00311, #E00312
- Deoxyribonuclease I (RNase free) #EN0521, #EN0523
#EN0525
- T7 Transcription Kit #K0411, #K0412
- RNA Ladder, Low Range #SM0411
- RNA Ladder, Low Range, ready-to-use #SM0413
- RNA Ladder, High Range #SM0421
- RNA Ladder, High Range, ready-to-use #SM0423
- NTP Set #R0481
- DEPC-treated Water #R0601, #R0603

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.