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T7 RNA Polymerase, GMP-Grade

Catalog #GMP-T7P-EE101

Storage Condition -20°C ± 5°C for 24 months. Avoid repeated freeze/thaw cycles.
Form Liquid
Source An *E. coli* strain that carries the gene for bacteriophage T7 RNA Polymerase
Storage Buffer 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 20 mM 2-mercaptoethanol, 0.1% Triton X-100, 50% Glycerol, pH 7.9
Concentration 50U/µL
Unit Definition One unit is defined as the amount of enzyme required to incorporate 1nmol ATP into acid-soluble material in a total reaction volume of 50µL in 1 hour at 37°C.

Product Contents

- T7 RNA Polymerase (50U/µL)
- 5X Transcription Buffer-1 (Tris-Acetate, $Mg(OAc)_2$, NaOAc, Spermidine, DTT, pH 8.1)

Product Description

T7 RNA Polymerase is a DNA-dependent RNA polymerase that is highly specific for the T7 phage promoters. T7 RNA Polymerase catalyzes *in vitro* mRNA synthesis in the 5' to 3' direction and accepts modified nucleotides into the transcripts. The mRNA products can be used for many downstream applications.

Applications

- mRNA for *in vitro* translation
- Radiolabeled RNA probe
- Non-isotopic RNA labeling
- RNA vaccines preparation
- Guide RNA preparation
- Antisense RNA preparation
- Capped mRNA synthesis with cap analog

Quality Control Statement

This product has been filed with the FDA Drug Master Files and is assigned DMF #037660. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

Quality Control Release Criteria

Assay	Criteria
Activity (Probe Incorporation)	≥ 50kU/mL
Purity (SEC-HPLC)	≥ 95%
Residual Nickel Salt	≤ 10 ppm
Endotoxin	≤ 10EU/mL
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Host Cell DNA l Host Cell DNA	≤ 100pg/mg
Residual Heavy Metal	≤ 10ppm
Bioburden	≤ 1CFU/10mL

Protocol for *In Vitro* Transcription

1. Prepare the following reaction mixture.

Reagent	Quantity
5X Transcription Buffer-1 (included with T7 RNA Polymerase)	4µL
CTP/GTP/ATP/UTP (100mM each)	2µL each
Murine RNase Inhibitor (120U/µL)	0.5µL
Pyrophosphatase, Inorganic (0.1U/µL)	1µL
T7 RNA Polymerase (50U/µL)	2µL
Template DNA	1µg
RNase-free Water	Up to 20µL

2. Incubate at 37°C for 1-2 hours.
3. After transcription, add 2U DNase I to remove DNA template for 15 minutes at 37°C.
4. Inactivate DNase I by phenol/chloroform extraction.

Notes

- Murine RNase Inhibitor is added to protect RNA from possible RNase contamination.
- The reaction mixture should be prepared at room temperature as DNA may precipitate in the presence of spermidine at 4°C.
- The reaction may be scaled up as needed.