E. coli RNA Polymerase, Holoenzyme

1-800-632-7799
info@neb.com
www.neb.com

M0551S 003170319031

M0551S

37°

50 units **Lot: 0031703** **Exp: 3/19**

1,000 U/ml **Store at –20°C**

Description: *E. coli* RNA Polymerase, Holoenzyme is the core enzyme saturated with sigma factor 70. The Holoenzyme initiates RNA synthesis from sigma 70 specific bacterial and phage promoters.

E. coli RNA Polymerase, Core Enzyme consists of 5 subunits designated α, α, β´, β, and ω. The enzyme is free of sigma factor and does not recognize any specific bacterial or phage DNA promoters. The enzyme retains the ability to transcribe RNA from nonspecific initiation sequences. Addition of sigma factors will allow the enzyme to initiate RNA synthesis from specific bacterial and phage promoters. The core enzyme has a molecular weight of approximately 400 kDa.

Source: *E. coli* RNA Polymerase, Holoenzyme is isolated from *E. coli* strain BL21. The sigma factor 70 is purified from an *E. coli* strain carrying the cloned gene for sigma factor 70.

Applications:

- RNA synthesis from *E. coli* promoter
- Transcription initiation studies
- *In vitro* translation with PURExpress

Supplied in: 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT) and 50% glycerol

Reagents Supplied with Enzyme:
5X *E. coli* RNA Polymerase Reaction Buffer

Reaction Conditions: 1X *E. coli* RNA Polymerase Reaction Buffer, supplemented with 0.5 mM each NTP and DNA template. Incubate at 37°C.

1X *E. coli* RNA Polymerase Reaction Buffer:
40 mM Tris-HCl
150 mM KCl
10 mM $MgCl_2$
1 mM dithiothreitol
0.01% Triton X-100™
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol NTP into RNA in 10 minutes at 37°C.

Unit Assay Conditions: 1X *E. coli* RNA Polymerase Reaction Buffer, supplemented with 0.5 mM of each NTP and 1 µg T7 phage DNA in 50 µl.

Quality Assurance: *E. coli* RNA Polymerase, Holoenzyme is free of detectable DNA endonuclease, exonuclease and RNase activities.

Quality Control Assays

DNA Endonuclease Activity: Incubation of a 50 µl reaction containing 5 units of *E. coli* RNA Polymerase, Holoenzyme with 1 µg of φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II, as determined by agarose gel electrophoresis.

RNase Assay: Incubation of a 10 µl reaction containing 1 unit of *E. coli* RNA Polymerase, Holoenzyme with 40 ng of RNA transcript for 4 hours at 37°C resulted in no detectable degradation, as determined by gel electrophoresis.

DNA Exonuclease Activity: Incubation of a 50 µl reaction containing 5 units of *E. coli* RNA Polymerase, Holoenzyme with 1 µg of a mixture of single- and double-stranded ^3H *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

(see other side)

CERTIFICATE OF ANALYSIS

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Companion Products Sold Separately:

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

RNase Inhibitor, Human Placenta
#M0307S 2,000 units
#M0307L 10,000 units

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

PURExpress® *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Companion Products Sold Separately:

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

RNase Inhibitor, Human Placenta
#M0307S 2,000 units
#M0307L 10,000 units

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

PURExpress® *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions

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