Poly(U) Polymerase

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

M0337S 003140916091

M0337S

RR NEB2 37° 65

60 units	**2,000 U/ml**	**Lot: 0031409**
RECOMBINANT	**Store at –20°C**	**Exp: 9/16**

Description: Poly(U) Polymerase catalyzes the template independent addition of UMP from UTP or AMP from ATP to the 3´ end of RNA.

Source: An *E. coli* strain that carries the cloned poly(U) polymerase gene of *Schizosaccharomyces pombe* Cid1.

Applications:
- Labeling of RNA with UTP
- Poly(U) tailing of RNA for cloning
- Studying effects of poly(U) tailing on stability and translation of RNA transferred into eukaryotic cells
- Poly(A) tailing of 2´O-Me modified 3´ ends

Supplied in: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1.0 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2

Reaction Conditions: 1X NEBuffer 2 supplemented with 1 mM UTP.* Incubate at 37°C.

Note: UTP is not included in the buffer.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme that incorporates 1 nmol of UMP into RNA in a 50 µl volume in 10 minutes at 37°C.

Unit Assay Conditions: 1X NEBuffer 2, 0.5 mM ^{3}H UTP and 5 µg yeast RNA are combined in a 50 µl reaction incubated at 37°C for 10 minutes.

Protocol for a Typical Tailing Reaction:

1. Combine the following in a sterile microcentrifuge tube:

10X NEBuffer 2	2.5 µl
UTP	0.5 mM final
RNA	>100 pmol
RNase Inhibitor* (40 units/µl)	1 µl
Poly(U) Polymerase (2 units/µl)	1 µl
H_2O	to 25 µl

2. Incubate at 37°C for 10 minutes.

*RNase Inhibitor is recommended but not required.

Quality Assurance: Poly(U) Polymerase contains no detectable DNAses, and RNAses. The purified protein contains no detectable DNA or RNA.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 2 units of Poly(U) Polymerase with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

DNA Exonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Poly(U) Polymerase with 1 cg of a mixture of single and double-stranded ^{3}H *E.coli* DNA for 3 hours at 37°C released < 0.1% of the total radioactivity.

DNA Endonuclease Activity: Incubation of a 50 µl reaction containing 10 units of Poly(U) Polymerase with 1 µg of supercoiled plasmid for 4 hours at 37°C resulted in < 10% conversion to nicked molecules as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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Notes: Poly(U) Polymerase in NEBuffer 2 will incorporate UMP or AMP from UTP or ATP into RNA. Tailing length of poly(U) varies with UTP. Poly(U) Polymerase is highly processive under low primer concentrations (< 100 pmol)

References:

1. Wickens, M. and Kwak, J.E. (2008) *Science* 319, 1344.
2. Kwak, J.E. and Wickens, M. (2008) *RNA* 13, 860.
3. Rissland, O.O.S., Mikulasova, A. and Norbury, C.J. (2007) *Molecular and Cell Biology* 27, 3612.

Companion Products:

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

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

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