

## Poly(U) Polymerase



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



M0337S 003130115011

# M0337S



60 units 2,000 U/ml Lot: 0031301

RECOMBINANT Store at -20°C Exp: 1/15

**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<sub>2</sub>  
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 <sup>3</sup>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 <sub>2</sub> O	to 25µl

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 <sup>3</sup>H *E. coli* DNA (200,000 cpm/µg) 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.

**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)

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

**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:**

Murine RNase Inhibitor

#M0314S 3,000 units

#M0314L 15,000 units

RNase Inhibitor

#M0307S 2,000 units

#M0307L 10,000 units

**References:**

1. Wickens, M. and Kwak, J.E. (2008) *Science* 319, 1344.
2. Kwak, J.E. and Wickens, M. (2008) *RNA* 13, 860.
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