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.

**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.

**Reaction Conditions:** 1X NEBuffer 2, 0.5 mM 3H 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
   - UTP 0.5 mM final
   - RNA
   - RNase Inhibitor* (40 units/µl) 1 µl
   - Poly(U) Polymerase (2 units/µl) 1 µl
   - H₂O 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.

**DNA Exonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of Poly(U) Polymerase with 1 µg of a mixture of single and double-stranded 3H *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.

**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 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).

**References:**

**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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