Poly(U) Polymerase

**Labeling of RNA with UTP**

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

**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.

**Reagents Supplied with Enzyme:**
- 1X NEBuffer 2
- Poly(U) Polymerase (2 units/µl)
- RNase Inhibitor* (40 units/µl)
- H2O

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

**Note:** UTP is not included in the buffer.

**Unit Assay 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
   - 2.5 µl UTP
   - 0.5 mM final RNA
   - 1 µl RNase Inhibitor* (40 units/µl)
   - 1 µl Poly(U) Polymerase (2 units/µl)
   - 1 µl H2O
   - to 25µl

2. Incubate at 37°C for 10 minutes.

**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 µg of a mixture of single and double-stranded ³H *E. coli* RNA (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).
References:

Companion Products:
Murine RNase Inhibitor
#M0314S 3,000 units
#M0314L 15,000 units

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