**T7 RNA Polymerase**  
**Tested User Friendly™**  
**Product numbers 70001/70047**  

**Brief protocol**

T7 RNA Polymerase is a single-subunit enzyme produced by bacteriophage T7. It is highly specific for T7 promoter and terminator sequences and it has been widely used for *in vitro* synthesis of specific RNAs. The transcripts may be directly used as substrates for studies of RNA structure or metabolism. If the transcripts are suitably labeled, they can also be used as sensitive hybridization probes. T7 RNA Polymerase may also be used to generate capped mRNA for expression studies in cells.

**Properties:**
- Molecular Weight: 98.8 KDa
- Optimum pH: 7.7–8.3
- Optimum temperature: 37°C
- Requirement for divalent cation: Mg<sup>2+</sup>
- Requirement of reducing agent: DTT, 2-mercaptoethanol
- Inhibitors: NaCl, KCl, or NH₄Cl above 75 mM
- Michaelis Constant: 40 µM for ATP, 160 µM for GTP, 60 µM for UTP, and 80 µM for CTP
- Inactivation: Incubation at 75°C for 10 minutes, or adding 1 µl of 0.5 M EDTA per 50 µl reaction

**10X T7 RNA Polymerase Transcription Buffer (included with the enzyme):**
- 400 mM Tris-HCl, pH 8.0
- 60 mM MgCl₂
- 100 mM DTT
- 100 mM NaCl
- 20 mM spermidine

T7 RNA Polymerase and 10X T7 RNA Polymerase Transcription Buffer have been functionally tested in the following protocol:

**In Vitro Transcription with T7 RNA Polymerase:**

1. For a 50 µl reaction add the following:
   - 10X T7 RNA Polymerase Transcription Buffer 5 µl
   - Ribonuclease Inhibitor (PN 71571) 10 units
   - ATP, 10 mM 5 µl
   - CTP, 10 mM 5 µl
   - GTP, 10 mM 5 µl
   - UTP*, 10 mM 5 µl
   - Linearized DNA template containing T7 promoter 1 µg
   - T7 RNA Polymerase 10–20 units
   - RNase-Free Water (PN 70783) to 50 µl

2. Incubate at 37°C for 1–2 hours.
3. Stop reaction by adding 1 µl of 0.5 M EDTA or by incubating at 75°C for 10 minutes.

*Note:* Either [α-<sup>32</sup>P]-UTP or [α-<sup>33</sup>P]-UTP may be used for preparing radiolabeled RNA probes.