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^{2+}
Requirement of Reducing Agent: DTT
Inhibitors: NaCl, KCl or NH_4Cl above 75mM
Michealis Constant: 47µM for ATP, 160µM for GTP, 60µM for UTP, and 81µM for CTP
Inactivation: Incubation at 75°C for 10 min, 1 µl of 0.5M EDTA per 50 µl reaction

10X T7 RNA Polymerase Transcription Buffer (PN 71100, included with the enzyme):
400mM Tris-HCl, pH 8.0, 150mM $MgCl_2$, 50mM DTT

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, 10mM	5 µl
CTP, 10mM	5 µl
GTP, 10mM	5 µl
UTP*, 10mM	5 µl
Linearized DNA 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 0.5M EDTA or by incubating at 75°C for 10 min.

***Note:** Either [α–^{32}P]-UTP or [α–^{33}P]-UTP may be used for preparing RNA probe.

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