

T7 Transcription Kit

T7 RNA Polymerase Kit

in vitro Transcription

	Cat. No.	Size	Conc.
	PCR-601S	10 reactions	50 units/ μ l
	PCR-601L	50 reactions	50 units/ μ l

For *in vitro* use only
 Quality guaranteed for 12 months
 Store at -20°C, avoid frequent thawing and freezing

T7 RNA Polymerase (red cap)
 50 units/ μ l

T7 Reaction Buffer (green cap)
 10x conc.

NTP Mix (white cap)
 10 mM of each NTP (ATP, CTP, GTP and UTP)

T7 Control Template (white cap)
 200 ng/ μ l, 1423 bp (including T7 promoter sequence)

RNase Inhibitor (yellow cap)
 40 units/ μ l RNase inhibitor in storage buffer with 50% glycerol (v/v)

RNase free water (white cap)

Description

T7 Transcription Kit provides optimized reagents for *in vitro* transcription by T7 RNA polymerase. The kit contains all reagents (including control template) to obtain high yields of RNA within a minimum reaction time. It guarantees efficient transcription of DNA templates containing a T7 promoter site.

Applications

- *In vitro* RNA synthesis
- RNA probe synthesis
- Generation of RNA template for *in vitro* translation
- RNAi (RNA interference) study

Template DNA

In general, any DNA (linearized plasmid DNA, PCR products) containing a T7 promoter site can be used as template for T7 RNA polymerase.

Minimum promoter sequence for efficient transcription:

5'-TAATACGACTCACTATAGGGAGA...-3'

└─┬─> Start of transcription

Recommended protocol

1. Assay preparation

Add the following components to a nuclease-free microtube and vortex gently:

Component	stock conc.	final conc.	1 assay
T7 Reaction Buffer	10x	1x	2 μ l
NTP Mix	10 mM each	500 μ M	1 μ l
RNase Inhibitor ¹⁾	40 units/ μ l	0.5-1 unit/ μ l	0.25-0.5 μ l
Template DNA with T7 promoter ²⁾		5-25 ng/ μ l	100-500 ng
T7 RNA Polymerase	50 units/ μ l	2.5 units/ μ l	1 μ l
nuclease-free water			fill up to 20 μ l

1) Addition of 10-20 units RNase inhibitor per 20 μ l assay is recommended (and may be essential when working with low amounts of template DNA)

2) For lower amounts of template DNA (<100 ng) an increased incubation time of up to 60 min is recommended.

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2. Incubation

Incubate the mixture at 37°C for 30 min.

Please note: Incubation at 42°C may increase the yield by about 10%. For lower amounts of template DNA (<100 ng) an increased incubation time of up to 60 min is recommended.

3. Optional DNase treatment

Add 1 µl (1 MBU) RNase-free DNase to the reaction product and incubate for 15 min at 37°C to remove template DNA.

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