

## SP6 RNA Polymerase

### RNA Polymerase

#### *in vitro* Transcription

	Cat. No.	Size	Conc.
	PCR-604S	2,000 units	50 units/ $\mu$ l
	PCR-604L	10,000 units	50 units/ $\mu$ l

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

#### Template DNA

In general, any DNA (linearized plasmid DNA, PCR products) containing a SP6 promoter site can be used as template for SP6 RNA polymerase.

*Minimum promoter sequence for efficient transcription:*

5'-ATTAGGTGACACTATAGAA**GA**...-3'

↳ Start of transcription

#### SP6 RNA Polymerase (red cap)

50 units/ $\mu$ l

#### SP6 Reaction Buffer (green cap)

10x conc.

#### Description

SP6 RNA polymerase is a DNA-dependent RNA polymerase that catalyzes the synthesis of RNA starting from a SP6 promoter sequence. The enzyme guarantees efficient transcription of DNA templates containing a SP6 promoter site.

#### Applications

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

#### Recommended protocol

##### 1. Assay preparation

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

Component	stock conc.	final conc.	1 assay
SP6 Reaction Buffer	10x	1x	2 $\mu$ l
NTP Mix	10 mM each	500 $\mu$ M	1 $\mu$ l
RNase Inhibitor <sup>1)</sup>	40 units/ $\mu$ l	0.5-1 unit/ $\mu$ l	0.25-0.5 $\mu$ l
Template DNA with SP6 promoter <sup>2)</sup>		5-25 ng/ $\mu$ l	100-500 ng
SP6 RNA Polymerase	50 units/ $\mu$ l	2.5 units/ $\mu$ l	1 $\mu$ l
nuclease-free water			fill up to 20 $\mu$ l

1) Addition of 10-20 units RNase inhibitor per 20  $\mu$ 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.

##### 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  $\mu$ l (1 MBU) RNase-free DNase to the reaction product and incubate for 15 min at 37°C to remove template DNA.