



## T7 P&L RNA Polymerase HC

Highly concentrated and modified T7 RNA Polymerase for set-up of high yield *in vitro* transcription reactions

Cat. No.	Amount
RNT-018	50 µl (2 µg/µl)

**For general laboratory use.**

**Shipping:** shipped on gel packs

**Storage Conditions:** store at -20 °C

**Additional Storage Conditions:** avoid freeze/thaw cycles

**Shelf Life:** 12 months

**Purity:** ≥ 95 % (SDS-PAGE)

**Form:** liquid

**Concentration:** 2.0 – 2.2 µg/µl (A280,  $\epsilon = 140 \text{ L mmol}^{-1} \text{ cm}^{-1}$  [1] in T7 RNA Polymerase HC Storage Buffer)

### Description:

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase (99 kDa) that catalyzes *in vitro* RNA synthesis from a DNA sequence containing a T7 phage promoter.

The highly concentrated version and modified T7 RNA Polymerase HC is ideally suited for set-up and optimization of high yield *in vitro* transcription reactions. Proline 266 has been replaced by leucine (P266L) which has been associated with

- decreased abortive transcription<sup>[1]</sup>.
- increased 5' homogeneity of transcripts synthesized from A-initiating phi2.5 promoter<sup>[2]</sup>
- increased 5' incorporation efficiency of GTP analogs<sup>[3]</sup>

The provided T7 IVT Set-up Buffer, MgCl<sub>2</sub> and DTT solutions enable testing of different reaction conditions such as nucleotide/ MgCl<sub>2</sub> concentrations. Recommendation for assay set-up with minimal optimization: HighYield T7 P&L RNA Synthesis Kit (#RNT-201).

**Activity:** 1 µl T7 P&L RNA Polymerase HC generates  $\geq 30$  µg RNA in 10 min at 37°. This is comparable to 1000 U of NEB's T7 RNA Polymerase (High Concentration), #M0460T (activity assay conditions: 1x T7 IVT Set-up Buffer supplemented with 10 mM DTT, 12 mM MgCl<sub>2</sub>, 3 mM NTPs, 1 µl T7 RNA Polymerase HC and 1 µg DNA template (1.4 kpb RNA transcript) in 20 µl. RNA yield was determined with a fluorescence microplate assay.)

### Content:

#### T7 P&L RNA Polymerase HC

1x 50 µl (2 µg/µl)  
50 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, 5 mM DTT, 0.1 % Triton-X-100, 50 % Glycerol (v/v), pH 8.0

#### T7 IVT Set-up Buffer

1x 200 µl (10x)  
400 mM Tris-HCl (pH 8.0), 20 mM Spermidin

#### DTT

1x 100 µl (100 mM)

#### MgCl<sub>2</sub>

1x 200 µl (200 mM)

### Related Products:

HighYield T7 RNA Synthesis Kit, #RNT-101  
RNase Inhibitor - recombinant, #PCR-392  
NTP bundle, #NU-1014

### Selected References:

[1] Guillerez *et al.* (2005) A mutation in T7 RNA polymerase that facilitates promoter clearance. *Natl. Acad. Sci. U.S.A* **102**:5958.