

P266L T7 RNA Polymerase Transcription Kit

Cat. No. PL71225

The **P266L T7 RNA Polymerase Transcription Kit*** produces high specific activity radiolabeled RNA probes (labeled-NTP provided by the user) from double-stranded DNA templates containing a phage T7 transcription promoter. The P266L T7 RNA polymerase included in the kit is a mutant form of phage T7 RNA polymerase first described by Guillerez, *et al.*¹ The P266L mutation significantly decreases abortive transcription initiation events and the concomitant production of short 5- to 8-nucleotide abortive RNA transcripts that can occur with the wild-type T7 RNA polymerase.

Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Enzyme Solution: contains P266L T7 RNA Polymerase at a concentration of 10 U/ μl as well as an RNase inhibitor.

Storage Buffers: P266L T7 RNA Polymerase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton[®] X-100, and 1 mM dithiothreitol (DTT). RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM CaCl_2 , and 10 mM MgCl_2 .

Unit Definitions: One unit of P266L T7 RNA polymerase converts 1 nmole of ribonucleoside triphosphates (NTPs) into acid-insoluble material in 60 minutes at 37°C . One Molecular Biology Unit (MBU) of RNase-Free DNase I converts 1 μg of pUC19 DNA into oligodeoxynucleotides in 10 minutes at 37°C .

5X Transcription Buffer: is 200 mM Tris-HCl (pH 7.5), 30 mM MgCl_2 , 50 mM NaCl, and 10 mM spermidine. DTT and NTPs must also be added to the final reaction.

Control Template: The control template is a linearized plasmid that will produce an 84 b runoff transcript. It contains an initially transcribed sequence (ITS) region which P266L T7 RNA polymerase can transcribe through much more efficiently than native T7 RNA polymerase.¹

P266L T7 RNA Polymerase Transcription Kit Contents

The kits provide sufficient reagents to perform 25 standard *in vitro* transcription reactions. Each kit contains the following:

P266L T7 RNA Polymerase Enzyme Solution (contains an RNase inhibitor).....	35 μl
P266L 5X Transcription Buffer.....	140 μl
100 mM Dithiothreitol.....	55 μl
10 mM ATP, CTP, GTP, and UTP Solutions.....	each at 35 μl
RNase-Free Water.....	300 μl
P266L T7 Control Template DNA @ 0.5 $\mu\text{g}/\mu\text{l}$	5 μg
RNase-Free DNase I @ 1 MBU/ μl	30 μl
5 M Ammonium Acetate Solution.....	275 μl

Quality Control: P266L T7 RNA Polymerase is function-tested by performance of a 50 μl non-labeled *in vitro* transcription reaction using 1 μg of control template in 1X Transcription Buffer at 37°C for 1 hour.

Contaminating Activity Assays: All of the components of the P266L T7 RNA Polymerase Transcription Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

References:

1. Guillerez, J. *et al.*, (2005) *Proc. Natl. Acad. Sci. USA* **102**, 5958.
2. Schenborn, E.T. and Mierendorf, R.C. (1985) *Nucl. Acids Res.* **13**, 6223.
3. Sambrook, J. *et al.*, (1989) *Molecular Cloning: A Laboratory Manual (2nd ed.)*, New York, Cold Spring Harbor Laboratory Press.
4. Milligan, J.F. *et al.*, (1987) *Nucl. Acids Res.* **15**, 8783.
5. Hoffman, L.M. and Johnson, M.G. (1994) *BioTechniques* **17**, 372.

* See page 3 for patent and licensing information.

-continued
Lit. #279

Notes on Using the P266L T7 RNA Polymerase Transcription Kit

1. Template Preparation: Transcription templates should be linear double-stranded DNA with blunt or 5'-protruding ends. Templates containing 3'-protruding ends can produce spurious transcripts due to non-specific initiation.² 3'-protruding ends can be readily converted to blunt ends with T4 DNA Polymerase.³ PCR products can also be used as templates, provided that the phage T7 promoter is present in the amplified sequence or has been incorporated into one of the primers used in amplification. Single-stranded DNA can also be used as template, however, the promoter region must be double-stranded. This can be accomplished by annealing a complementary oligonucleotide to the promoter region before transcription.^{4,5}

The quality of the DNA template directly affects the quality of the RNA produced. Generally, DNA is of sufficient quality for use as a transcription template if it is free of contaminating RNase and can be fully digested with restriction enzymes. To confirm that a template is fully linearized and intact, examine the DNA on an ethidium-stained agarose or polyacrylamide gel prior to use in a transcription reaction.

Templates that give low yields or less than full-length transcripts may contain RNase or other contaminants. Such templates will usually give better results after the following treatment:³

- 1) Add Proteinase K to 100-200 µg/ml and SDS to 0.5%.
- 2) Incubate for 30-60 minutes at 37°C.
- 3) Extract with an equal volume of a 1:1 mixture of TE-saturated phenol/chloroform.
- 4) Ethanol precipitate.
- 5) Gently remove the supernatant and rinse the pellet with 70% ethanol.
- 6) Resuspend at 1.0 µg/µl in RNase-Free T₁₀E₁ (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

In some cases, increasing the reaction temperature from 37°C to 42°C may also improve the yield.

2. Alternative to Ethanol Precipitation: For transcripts ≥100 bases, we recommend purifying the RNA by the simple addition of one volume of 5 M ammonium acetate followed by centrifugation. This method selectively precipitates RNA while leaving most of the DNA, protein, and unincorporated NTPs in the supernatant. The protocol is:

- 1) Add one volume of 5 M ammonium acetate (20 µl for the typical reaction).
- 2) Incubate for 15 minutes on ice.
- 3) Centrifuge at high speed (~10,000 x g) for 15 minutes at 4°C.
- 4) Wash the pellet in 70% ethanol.
- 5) Resuspend the pellet in RNase-Free H₂O or T₁₀E₁.

Related Products: The following products are also available:

- RiboScribe™ RNA Probe Synthesis Kits
- AmpliScribe™ T7-Flash™ Transcription Kit
- AmpliScribe™ T7, T3 and SP6 High Yield Transcription Kits
- MessageMAX™ T7 ARCA-Capped Message Transcription Kit
- AmpliCap-Max™ T7 & T3 High Yield Message Maker Kits
- AmpliCap™ T7, T3, and SP6 High Yield Message Maker Kits
- RNA Cap Analogs
- ScriptCap™ m⁷G Capping System
- ScriptCap™ 2'-O-Methyltransferase
- Poly(A) Polymerase Tailing Kit
- T7, T3, and SP6 Phage RNA Polymerases
- RNase-Free DNase I
- AmpliScribe™ T7 Aminoallyl-RNA Transcription Kit
- DuraScribe™ T7 Transcription Kit
- T7 and SP6 R&DNA™ Polymerases
- NTP Solutions

Standard Transcription Reaction: Synthesis of Radiolabeled RNA

- Combine the following reaction components at room temperature in the order given:
(see Notes below)

	<u>Final Concentration</u>
x μ l RNase-Free water	---
1 μ g linearized template DNA with T7 promoter	50 ng/ μ l
4 μ l P266L 5X Transcription Buffer	1X
1 μ l 10 mM ATP	0.5 mM
1 μ l 10 mM GTP	0.5 mM
1 μ l 10 mM UTP	0.5 mM
3 μ l 100 μ M CTP (1:100 dilution of the 10 mM CTP stock in RNase-Free H ₂ O)	15 μ M
2 μ l 100 mM DTT	10 mM
5 μ l α -[³² P]-CTP (400 Ci/mmol, 50 μ Ci)	2.5 μ Ci/ μ l
1 μ l P266L T7 RNA Polymerase Enzyme Solution	0.5 U/ μ l
<hr/>	
20 μ l Total reaction volume	

- Incubate at 37°C for 2 hours.
- (Optional) Add 1 μ l (1 MBU) of RNase-Free DNase I and incubate for 15 minutes at 37°C.
- (Optional) Labeled RNA may be concentrated by ammonium acetate precipitation (see "Alternative to Ethanol Precipitation", page 2) or by standard ethanol precipitation procedures.

Notes

Reaction Volume: The volume of this reaction may be scaled up or down proportionately.

Choice of Radionucleotide: The above protocol uses α -[³²P]-CTP as the radiolabel, however, any α -[³²P]-NTP accompanied by the appropriate non-labeled NTP dilution may be substituted.

Probe Specific Activity: If probes of higher specific activity are desired, they may be synthesized by directly substituting a higher specific activity radionucleotide (800 Ci/mmol) into the above reaction. Probes generated with higher activity radionucleotide are more susceptible to radiolytic cleavage and are thus useful for a shorter period of time. Note however, that probes synthesized with radiolabel of 400 Ci/mmol are sufficient for most applications.

DNase Treatment: It may not be necessary to remove the template DNA or unincorporated label before proceeding to subsequent experiments.

Synthesis of Non-Radioactive RNA: Non-radioactive RNA may be synthesized by substituting 1 μ l of the 10 mM CTP stock solution in place of the α -[³²P]-CTP and 1:100 dilution of non-labeled CTP in the above protocol. For synthesis of up to 180 μ g of non-radioactive RNA including fluorescent-, biotinylated- or digoxigenin-labeled RNA, use the appropriate AmpliScribe or AmpliScribe *Flash* High Yield Transcription Kit.

Triton is a registered trademark of Rohm & Haas, Philadelphia, Pennsylvania.

RiboScribe, AmpliScribe, T7-Flash, MessageMAX, AmpliCap-Max, AmpliCap, ScriptCap, DuraScribe, and R&DNA are trademarks of EPICENTRE, Madison, Wisconsin.

The **P266L T7 RNA Polymerase Transcription Kit** is covered by U.S. Patent No. 7,335,471, European Patent No. 1385963, and by other patents issued or pending licensed to EPICENTRE. This product is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for life science research. Contact EPICENTRE for information on licenses to other uses.