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T7 RNA Polymerase

For all sizes of T7 RNA Polymerase
(Cat. Nos. T7905K, T7950K, TM910K, TH950K, TU950K)

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1. Introduction

In vitro transcription with T7 RNA Polymerase enables the synthesis of specific RNA from DNA sequences cloned downstream of the appropriate promoter. RNA can be radiolabeled for use as a probe in various blotting and *in situ* hybridization techniques. Nonradioactive RNA synthesized with T7 RNA Polymerase can be used in studies of translation, RNA processing, and antisense RNA.

2. Product Specifications

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

Storage Buffer: T7 RNA Polymerase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, and 0.1% Triton® X-100.

Unit Definition: One unit converts 1 nmol of ribonucleoside triphosphates (NTPs) into acid-insoluble material in 60 minutes at 37°C.

5X Transcription Buffer: 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.

Activity Assay: The unit assay is performed in a reaction containing 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 5 µg DNA template, 0.5 mM of each NTP, and varying amounts of enzyme.

Contaminating Activity Assays: T7 RNA Polymerase is free of detectable DNA exonuclease and endonuclease, RNase, and *E. coli* RNA polymerase activities.

3. Example Protocol

3.A. Standard Transcription Reaction: Synthesis of Nonradioactive RNA

1. Combine the following reaction components at room temperature in the order given:

			Final Concentration
x	µl	RNase-Free water	---
0.5-1	µg	linearized template DNA with appropriate promoter	25-50 ng/µl
4	µl	5X Transcription Buffer	1X
1	µl	10 mM ATP	0.5 mM
1	µl	10 mM CTP	0.5 mM
1	µl	10 mM GTP	0.5 mM
1	µl	10 mM UTP	0.5 mM
2	µl	100 mM DTT	10 mM
10	U	T7 RNA Polymerase	0.5 U/µl
20	µl	Total reaction volume	

2. Incubate at 37°C for 2 hours.
3. (Optional) Add 1 µl (1 MBU) of RNase-Free DNase I and incubate for 15 minutes at 37°C.

The volume of this reaction may be increased or decreased proportionately.

3.B. Synthesis of Radioactive RNA

Radioactive RNA may be synthesized by adding a radioactive nucleoside triphosphate and decreasing the final concentration of the corresponding nonradioactive NTP to 15 µM. As the total concentration of the labeled NTP will be limiting, virtually all of the radioactivity will be incorporated into RNA. For example, to label with α-[^{32}P]-CTP, first prepare a stock of 100 µM CTP by diluting the 10 mM CTP stock 1 to 100 with RNase-Free H_2O. Then add 3 µl of the 100 µM CTP and the desired amount of α-[^{32}P]-CTP in place of the 10 mM CTP and some of the water in the above protocol.

4. Related Products

The following products are also available:

Cat. #	Concentration	Quantity
AmpliScribe™ T7-*Flash*™ Transcription Kit		
ASF3257		25 Reactions
ASF3507		50 Reactions
DuraScribe® T7 Transcription Kit		
DS010910		10 Reactions
DS010925		25 Reactions
RNase-Free DNase I		
D9902K	1 U/µl	2,500 MBU
D9905K	1 U/µl	5,000 MBU
D9910K	1 U/µl	10,000 MBU (2 x 5,000 MBU)

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