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T7 R&DNA™ Polymerase

Cat. Nos. D7P9201K and D7P9205K

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1. Introuction

T7 R&DNA™ Polymerase* and the corresponding wild-type T7 RNA polymerase are useful for *in vitro* synthesis of defined "transcripts" that are complementary to nucleic acids cloned into a plasmid or other vector downstream from a T7 RNA polymerase promoter. In contrast to the corresponding wild-type T7 RNA polymerase, the T7 R&DNA Polymerase can incorporate 2′-deoxyribonucleoside-5′-triphosphates or other 2′-modified triphosphates such as ribonuclease-resistant 2′-fluoro-ribonucleoside-5′-triphosphates, in addition to the canonical ribonucleoside-5′-triphosphates. The ability of this mutant enzyme to incorporate various non-canonical 2′-ribonucleotides permits either primed or unprimed in vitro synthesis of "transcripts" composed of rNMPs, dNMPs, modified 2′-NMPs, or of mixed dNMP/rNMP, or 2′-modified-NMP/rNMP composition for a variety of applications.

T7 R&DNA Polymerase is available in 1,000- and 5,000-Unit sizes at a concentration of 50 U/µl. Separate tubes of R&DNA Polymerase 5X Reaction Buffer and 100 mM dithiothreitol (DTT) solution are also provided.

2. Product Specifications

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

Storage Buffer: T7 R&DNA Polymerase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1.0 mM DTT, 0.1 mM EDTA, and 0.1% Triton® X-100.

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

R&DNA Polymerase 5X Reaction Buffer: 0.2 M 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.

Quality Control: T7 R&DNA Polymerase is function-tested for RNA polymerase activity and incorporation of dCTP in two independent reactions containing 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 1 or 5 µg linearized DNA template, 0.5 mM of each NTP or 0.5 mM of each ATP/GTP/UTP/dCTP and varying amounts of enzyme.

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

3. Related Products

The following products are also available:

- T7 Phage RNA Polymerase
- AmpliScribe™ T7-*Flash*™ Transcription Kit
- dNTP Solutions
- NTP Solutions
- RNase-Free DNase I

4. Suggested Reaction Protocol

T7 R&DNA Polymerase will incorporate many different 2′-deoxy NTPs and 2′-modified NTPs. The efficiency of incorporation, yield, and optimal reaction conditions are likely vary depending on the specific NTP substitution.

In general,

A. 2′-fluoro-dNTPs (2′-Fl-dNTPs) are incorporated more efficiently and produce higher yields of full-length chimeric RNA/DNA than the 2′-dNTPs.

B. Complete substitution of one 2′-Fl-dNTP (or one dNTP) for a rNTP in a T7 R&DNA Polymerase reaction results in a slight decrease in yield. Additional substitutions of NTPs will subsequently reduce yields of transcript produced. Substitution of all four NTPs will result in extremely low yields of transcript and is not recommended.

Protocol

1. Combine the following reaction components on ice in the order given:

x	µl	RNase-Free water
1	µg	linearized template DNA with appropriate promoter
4	µl	R&DNA Polymerase 5X Reaction Buffer
1	µl	of each 10 mM NTP or 2′-Fl-dNTP or dNTP or other modified-NTP or dNTP
2	µl	100 mM DTT
0.5	µl	T7 R&DNA Polymerase
20	µl	Total reaction volume

2. Incubate at 37°C for 2 hours.
3. Isolate or purify the reaction products by method of choice for use in subsequent experiments.

5. Related References

1. Sousa, R. and Padilla, R. (1995) *EMBO J.* **14** (18), 4609.
2. Padilla, R. and Sousa, R. (1999) *Nucl. Acids Res.* **27** (6), 1561.
3. Huang, Y. *et al.*, (1997) *Biochemistry* **36**, 8231.

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