Manual

T7 R&DNA Polymerase

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T7 R&DNA Polymerase is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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GENOMIC ANALYSIS BY LGC

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1. Introduction

T7 R&DNA Polymerase* and the corresponding wild-type T7 RNA polymerase are useful for i*n 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.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
T7 R&DNA Polymerase	5,000 units	D7P9205K	T7 R&DNA Polymerase (50 U/µL)	E0084-50D1	100 µL
			DTT (100 mM, dithiothreitol)	SS000065-D7	400 µL
			R&DNA Polymerase 5X Reaction Buffer	SS000333-D1	1 mL

3. 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 (Rohm & Haas)

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 linearised 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.

4. Suggested reaction protocol

T7 R&DNA Polymerase will incorporate many different 2′-deoxy NTPs and 2′-modified NTPs (except for 2′-O-methyl UTP or CTP). 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 linearised 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.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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