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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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T7 RNA Polymerase (200 U/μL)

Cat no. LDG0001RI

Product Overview

Package component

Item	Amount
T7 RNA Polymerase (200 U/μL)	25,000 U
10X RNA Polymerase reaction buffer	1 vial (1 mL)
100 mM DTT	1 vial (1 mL)

Description

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase with high specificity for the T7 promoter. This enzyme catalyzes the 5′ →3′ synthesis of RNA from DNA downstream from its promoter.

Source

Escherichia coli

Activity

One unit of T7 RNA Polymerase is defined as the amount of the enzyme incorporates 1 nmol of ATP into acid-insoluble product in 1 hour at 37°C.

Storage buffer

The enzyme is supplied in 100 mM Tris-HCl (pH 7.9), 20 mM KCl, 1 mM DTT, 1 mM EDTA, 0.1% Triton® X-100 and 50% (v/v) glycerol.

10X RNA Polymerase Reaction Buffer

400 mM Tris-HCl (pH 8.0), 60 mM MgCl2, and 20 mM spermidine.

Storage and Stability

Stored at -20°C. For optimal storage, aliquot the enzyme, reaction buffer and DTT reagent into smaller quantities and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Procedure

Standard RNA synthesis procedures:

1. Below reaction mixture should be prepared under room temperature and combined in the following order:

Component	Amount	Final concentration
Nuclease-Free H_2O	X μL	-
Template DNA	0.5-1 μg	-
10X RNA Polymerase Reaction Buffer	2 μL	1X
ATP (100 mM)	0.6 μL	3 mM
UTP (100 mM)	0.6 μL	3 mM
CTP (100 mM)	0.6 μL	3 mM
GTP (100 mM)	0.6 μL	3 mM
100 mM DTT	2 μL	10 mM
T7 RNA Polymerase (200 U/μL)	1 μL	-
RNase inhibitor (optional)	0.5 μL	1 U/μL
Total reaction volume	20 μL	-

2. Incubate at 37°C for 30 minutes to 2 hours.
3. Above reaction mixture may be scaled up or down proportionately.

Important notes

(1) Transcription reaction should be performed under RNase free condition. Use nuclease-free tubes, reagents, and water to avoid RNase contamination. Also, wear gloves when working with RNA.

(2) To obtain optimal condition, NTP concentration can be titrated between 3 – 5 mM.

(3) The volume of T7 RNA Polymerase can be titrated between 1-2 μL in the IVT reaction to optimize your assay.

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Disclaimer

This product is for research use only and is not intended for diagnostic use.

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