PRODUCT INFORMATION

T7 RNA Polymerase

v. 230701

Catalog number C15010H-25000U

Set package & Component

Cat.	Name	Amount
C15010H-25000U	T7 RNA Polymerase (200 U/μL)	25,000 U
	10X RNA Polymerase reaction buffer	1 mL
	100 mM DTT	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*

Purity >98% as determined by SDS-PAGE analysis.

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

Reaction Condition 1X RNA Polymerase Reaction Buffer, supplemented with 3 mM each ATP, UTP, GTP, CTP, and DNA template containing the T7 RNA Polymerase promoter. Incubate at 37°C.
10X RNA Polymerase Reaction Buffer: 400 mM Tris-HCl (pH 8.0), 60 mM $MgCl_2$, and 20 mM spermidine.

Manuel

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.
Storage Buffer	T7 RNA Polymerase 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.
Storage	Stored at -20°C. ***For optimal storage, aliquot the 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.***
Notes	1. Transcription reaction should be performed under RNase free condition. Use nuclease-free tubes, reagents, and water to avoid RNase contamination. Also, 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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