PRODUCT INFORMATION

GMP T7 RNA Polymerase

v. 250301

Catalog number	C15010-GMP-25KU / C15010-GMP-200KU

Set package & Component

Cat.	Name	Amount
C15010-GMP-25KU	GMP T7 RNA Polymerase (200 U/μL)	25 KU
	GMP 10X RNA Polymerase reaction buffer	1 mL
	GMP 100 mM DTT	1 mL
C15010-GMP-200KU	GMP T7 RNA Polymerase (200 U/μL)	200 KU
	GMP 10X RNA Polymerase reaction buffer	4 mL
	GMP 100 mM DTT	4 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.
Endotoxin level	<0.05 EU per 1 μg of the protein by the LAL method.
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	Store at -20°C for up to 6 months, and it is recommended to store at -80°C for long-term preservation. Avoid repeated freeze/thaw cycles.
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.
Handling Instruction	For optimal storage, aliquot the enzyme, reaction buffer and DTT reagent into smaller quantities and store at recommended temperature. Avoid extended exposure to ice; instead, promptly retrieve the required portion and return it to the appropriate storage temperature.
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	
GMP 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
GMP 100 mM DTT	2 µL	10 mM
GMP 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.

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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