

PRODUCT INFORMATION

T7 RNA Polymerase with buffer C

v. 231001

Catalog number	C15010HC-25000U		
	Cat.	Name	Amount
Set package &	C15010HC-25000U	T7 RNA Polymerase (200 U/μL)	25,000 U
Component		10X RNA Polymerase reaction buffer C	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 $5'\rightarrow 3'$ 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.		
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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 H₂O	XμL	-
Template DNA	0.5-1 μg	
10X RNA Polymerase Reaction Buffer C	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	

Manuel



	 Incubate at 37°C for 30 minutes to 2 hours. 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	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.	
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.	
Notes	 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. 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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