

T7 RNA Polymerase with buffer D

v. 231001

Catalog number	C15010HD-25000U		
Set package & Component	Cat.	Name	Amount
	C15010HD-25000U	T7 RNA Polymerase (200 U/μL)	25,000 U
		10X RNA Polymerase reaction buffer D	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	<i>Escherichia coli</i>		
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.		
Manual	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	X μL	-
	Template DNA	0.5-1 μg	
	10X RNA Polymerase Reaction Buffer D	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	-	

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