

**T7 RNA Polymerase with buffer B**

v. 231001

<b>Catalog number</b>	C15010HB-25000U		
<b>Set package &amp; Component</b>	<b>Cat.</b>	<b>Name</b>	<b>Amount</b>
	C15010HB-25000U	T7 RNA Polymerase (200 U/μL)	25,000 U
		10X RNA Polymerase reaction buffer B	1 mL
		100 mM DTT	1 mL
<b>Description</b>	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.		
<b>Source</b>	<i>Escherichia coli</i>		
<b>Purity</b>	>98% as determined by SDS-PAGE analysis.		
<b>Unit Definition</b>	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.		
<b>Reaction Condition</b>	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.		
<b>Manual</b>	Standard RNA synthesis procedures:		
	1. Below reaction mixture should be prepared under room temperature and combined in the following order:		
	<b>Component</b>	<b>Amount</b>	<b>Final concentration</b>
	<b>Nuclease-Free H<sub>2</sub>O</b>	X μL	-
	<b>Template DNA</b>	0.5-1 μg	
	<b>10X RNA Polymerase Reaction Buffer B</b>	2 μL	1X
	<b>ATP (100 mM)</b>	0.6 μL	3 mM
	<b>UTP (100 mM)</b>	0.6 μL	3 mM
	<b>CTP (100 mM)</b>	0.6 μL	3 mM
	<b>GTP (100 mM)</b>	0.6 μL	3 mM
	<b>100 mM DTT</b>	2 μL	10 mM
	<b>T7 RNA Polymerase (200 U/μL)</b>	1 μL	-
<b>RNase inhibitor (optional)</b>	0.5 μL	1 U/μL	
<b>Total reaction volume</b>	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.

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**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.

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**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.

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- 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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