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INSTRUCTIONS FOR USE

Product Name: T7 RNA Polymerase, Low dsRNA, GMP-Grade (250 U/μL) **Catalog # T703-E51B**

Step 1. Remove all experimental materials from -20°C and place them on an ice pack to thaw. Mix thoroughly, briefly centrifuge to collect the liquid at the bottom of the tube and keep on ice for later use.

Step 2. Prepare the appropriate reaction system below and ensure that all reagents and containers are free of RNase contamination.

Standard Transcription System

2 μL (1x concentration)	10x Transcription Buffer
2 μL each (10mM each)	CTP / GTP/ ATP/ UTP (100 mM each)
As required	T7 RNA Polymerase (low dsRNA, 250 U/μL)
0.5 μL	Murine RNase Inhibitor (40 U/μL)
0.4 μL	Pyrophosphatase, Inorganic (0.1 U/μL)
Up to 20 μL	RNase free H20
X μg	DNA template

Co-transcriptional Capping System

2 μL (1x concentration)	10x Transcription Buffer
2 μL each (10mM each)	CTP / GTP/ ATP/ UTP (100 mM each)
2 μL (10mM)	Cap1-analog (100 mM)
1-1.6 μL	T7 RNA Polymerase (low dsRNA, 250 U/μL)
0.5 μL	Murine RNase Inhibitor (40 U/μL)
0.4 μL	Pyrophosphatase, Inorganic (0.1 U/μL)
Up to 20 μL	RNase free H20
X μL (1μg)	DNA template

- The reaction system can be scaled up proportionally to produce more RNA without affecting the reaction. In a 20 μL transcription system, approximately 180-240 μg of RNA can be obtained.
- For transcripts less than 100 nt: the template amount should be increased to 2 μg, and the transcription time can be extended to 4-8 hours.
- Use linearized plasmid templates for transcription to improve RNA quality.

Step 3. Incubate at 37°C for 2-3 hours.

Step 4. To degrade DNA template: Add 1 µL DNase I and incubate at 37°C for 15-30 minutes.

Step 5. Purify and check the quality of the synthesized RNA before continuing to the subsequent experiments.