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INSTRUCTIONS FOR USE

Product Name: T7 RNA Polymerase, GMP-Grade (250 U/µL) **Catalog # T702-E51B**

***In vitro* transcription without cap-analog:**

Step 1. In a centrifuge tube add the following reaction components:

2 µL (1x concentration)	10x Transcription Buffer
2 µL each (10mM each)	CTP / GTP/ ATP/ UTP (100 mM each)
5 µL	T7 RNA Polymerase (50 U/µL)
0.04 µL	Pyrophosphatase, Inorganic (1 U/µL)
0.5 µL	RNase inhibitor (40 U/µL)
Up to 18 µL	RNase free H20
2 µL (100ng-1µg)	DNA template

Note: The DNA template should be added last. The 10x Transcription Buffer contains high concentration of spermidine, which may cause precipitation of DNA templates.

- It is advisable to keep the water and buffer at room temperature before use. The reaction mix should also be prepared at room temperature because spermidine will cause precipitation of high-concentration DNA templates at low temperature.
- For transcripts less than 100 nt: the templates should be increased to 2µg.
- To ensure effective transcription of a specific region, it is recommended to cut the DNA template downstream region into blunt ends or 5' protruding ends.

Step 2. Incubate the reaction at 37°C for 2-4 h (For transcripts less than 100 nt: increase the incubating time to 4-8 h).

Step 3. To degrade the DNA template: Add 2U DNase I and incubate at 37°C for 15-30 min.

Step 4. Purification of transcripts: RNA Cleaner magnetic beads can be used to remove proteins, salt ions and other impurities. Phenol/chloroform purification method can also be used.

***In vitro* transcription with cap1-analog:**

Step 1. In a centrifuge tube add the following reaction components:

2 µL (1x concentration)	10x Transcription Buffer
2 µL each (10mM each)	CTP / GTP/ ATP/ UTP (100 mM each)
2 µL each (10mM each)	Cap1-analog (100 mM each)
5 µL	T7 RNA Polymerase (50 U/µL)
0.04 µL	Pyrophosphatase, Inorganic (1 U/µL)

0.5 µL	RNase inhibitor (40 U/µL)
Up to 18 µL	RNase free H20
2 µL (100ng-1µg)	DNA template

Step 2. Incubate the reaction at 37°C for 2-4 h.

Step 3. To degrade the DNA template: Add 2U DNase I and incubate at 37°C for 15-30 min.

Step 4. Purification of transcripts: RNA Cleaner magnetic beads can be used to remove proteins, salt ions and other impurities. Phenol/chloroform purification method can also be used.