MediRes Corp.

USER GUIDE **Bi2M-T7TK**

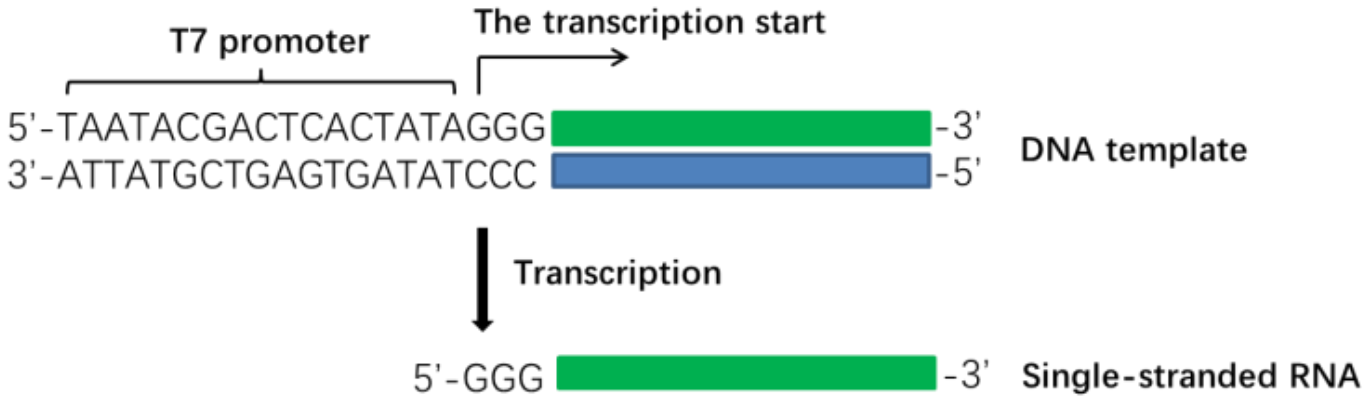
T7 High Yield Transcription Kit

Product #: Bi2M-T7TK

Quantity: 50 reactions

Product Description

- This kit uses T7 RNA Polymerase to transcribe and synthesize RNA in vitro.
- Templates can include linearized plasmid DNA with a T7 promoter, PCR products, or synthetic DNA.
- NTP is used as the substrate for the reaction.
- The kit optimizes the RNA transcription reaction system for efficient and rapid RNA production.
- Adding modified nucleotides during transcription allows preparation of biotin or dye-labeled RNA.
- Applications include in vitro translation, RNase protection experiments, hybridization probe labeling, RNA shearing, and other biological experiments.
- The reaction system can produce over 100 μg of RNA from 1 μg of template, suitable for various RNA lengths.
- Schematic diagram of T7 RNA polymerase transcription:



Storage

- Store at -20℃.

Components Provided

T7 RNA Transcription Enzyme Mix	200µL
5X T7 Transcription Reaction Buffer	250µL
25 mM NTP Mix	100µL
DNase I	50µL
Nuclease Free Water	1mL
Control Template (0.5µg/µL)	10µL

Protocol

Preparation of Template

A. Plasmid with T7 Promoter as Template:

1. Ensure the plasmid template is fully linearized (purified for use as template).
2. The linearized plasmid should have a blunt end or a 5' overhang (avoid 3' overhangs).
3. Recommended template amount per reaction: 1 µg.

B. PCR Product with T7 Promoter or Synthesized DNA Fragment as Template:

1. Add the T7 promoter sequence (5’-TAATACGACTCACTATAGGG-3’) to the 5' end of the non-coding strand primer during PCR amplification.
2. PCR products can be used directly as transcription templates without purification.
3. For higher RNA yield, purify the PCR product before use.
4. Recommended template amount per reaction: approximately 0.5 µg.

Transcription Reaction

1. Reaction Setup:
   A. Add reagents into a clean tube according to the reaction system recommended in the table.

B. Mix thoroughly and incubate at 37°C for 2 hours.
C. For RNA synthesis less than 300 nucleotides, extend the reaction time to 4 hours or longer.

COMPONENT	FINAL VOLUME/AMOUNT
Template	0.5-1μg
5X T7 Transcription Reaction Buffer	4μL
25 mM NTP Mix	2μL
T7 RNA Transcription Enzyme Mix	4μL
Nuclease Free Water	To 20μL

2. Post-Reaction Treatment:
   A. Add 1 μL DNase I to the reaction system.
   B. Incubate at 37°C for 15 minutes to digest the transcribed DNA template.
3. RNA Analysis and Purification:
   A. Analyze and purify the synthesized RNA using electrophoresis before downstream experiments. Alternatively, the RNA fraction can be purified using an RNA column purification kit.
4. Quantification and Detection of RNA:
   A. Determine RNA concentration using the UV absorption method (ensure RNA products are purified to avoid inaccuracies from free nucleotides).
   B. For electrophoresis detection, use a 1% formaldehyde agarose denaturing gel and 1×MOPS Buffer.
      - **MOPS Buffer Preparation:** 10×MOPS Buffer contains 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, and 10 mM EDTA.
      - **Gel Preparation:** Dissolve 0.5 g agarose in 36 mL RNase-free water. Heat and melt, then add 5 mL of 10×MOPS Buffer. Cool until

manageable and add 9 mL formaldehyde solution (37%). Mix well and pour the gel.

C. Mix an appropriate amount of RNA with RNA Loading Buffer.
D. Incubate at 70°C for 10 minutes, followed by an ice bath for 2 minutes, and collect all samples.
E. After electrophoresis, stain with EB or Safe-Red gel stain (Cat# **Bi2M-SrRed**) to observe the results.