

In vitro transcription

Materials:

- T7 High Yield RNA Transcription Kit (Vazyme, catalog no. TR101)
- Nuclease-free, barrier tips and pipets capable of pipetting $0-1000~\mu L$
- Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
- Nuclease-free, molecular biology-grade water

Procedure:

- 1. The components except T7 RNA Polymerase Mix were oscillated and mixed, briefly centrifuged and collected at the bottom of the tube, and stored on ice for later use.
- 2. The amount of DNA template is between 0.1-1ng. If the product is less than 300bp, it is suggested to increase the amount of DNA template to 2ug.

 Prepare reaction according to the following table:

_	_		
П	Γа	h	ام

10 x Reaction Buffer	2 μL	
ATP Solution	2 μL	
GTP Solution	2 μL	
CTP Solution	2 μL	
UTP Solution	2 μL	
DNA template	x μL	
T7 RNA Polymerase Mix	2 μL	
RNase-free ddH2O	up to 20µl	

- 3. The components were gently mixed with a pipette, collected by centrifugation for a short time, and incubated at 37°C for 2h.
- 4. Add $1\mu L$ DNase I to the reaction system, and incubate at $37^{\circ}C$ for 15min to digest the template.
- 5. The synthesized RNA was analyzed by electrophoresis and purified for downstream experiments.