T7 Transcription Kit User Guide

Product Description

This transcription kit features an optimized in vitro transcription system using T7 RNA Polymerase. It efficiently transcribes DNA sequences downstream of the T7 promoter from either supercoiled plasmid DNA or linear DNA templates containing a T7 promoter. This kit is suitable for producing high-concentration RNA transcripts longer than 6,000 nt. Using 1 μg of DNA template in a 20 μL reaction, 150-280 μg of RNA can be generated. For milligram-scale RNA production, the reaction can be scaled up proportionally.

The synthesized RNA is compatible with applications such as in vitro translation, RNase protection assays, RNA cleavage, and labeling of hybridization probes.

Storage Condition

Store at -20 °C for up to one year.

Kit Components

Component	EDN-T701(25rxns)	EDN-T702(100 rxns)
T7 Transcription Enzyme Mix	50μl	200μl
5×T7 Transcription Reaction Buffer	100μl	400μl
ATP(100mM)	50μl	200μl
GTP(100mM)	50μl	200μl
CTP(100mM)	50μl	200μl
UTP(100mM)	50μl	200μl
DNaseI(1 unit/ul)	50μl	200μl
500mM EDTA(pH 8.0)	25μl	100μl
RNase-free Water	1ml	5ml
Transcription Control Template(0.5μg/μl)	10μl	40μl

Template Reference

T7 Promoter：5'-TAATACGACTCACTATAGGG[#]-3'　　#:G/A

Terminator：5'TTCCATCTGTTTTCTTATCTGTTCTTTCATCTGTTCTTTTATCTGTTTGTTT 3'

Template Amount	RNA Yield
2μg	170~320μg
1 μg	150~280μg
500 ng	100~180μg
200 ng	40~80μg
100 ng	15~40μg
50ng	10~20μg
10ng	4~8μg
lng	2~6μg

- **Procedure**

1. Briefly centrifuge all components except the T7 Enzyme Mix and collect them at the bottom of the tube.
2. Prepare the transcription reaction as follows:

Component	Volume	Final Concentration
Template	1ng~2μg	NA
5×T7 Transcription Reaction Buffer	4μl	1×
A/G/C/UTP	1.6μl each	8mM each
T7 Transcription Enzyme Mix	2μl	NA
RNase-free Water	Variable	NA
Total Volume	20μl	20μl

Note: Before use, calculate the total reaction setup and strictly follow the order of addition: Water → Buffer → NTPs → DNA Template → Enzyme.

3. Gently mix all components using a pipette and briefly centrifuge to collect the reaction at the bottom of the tube. Incubate at 37 °C for 2 hours.

Note: To prevent evaporation from prolonged transcription, it is recommended to perform the reaction in a PCR machine with the heated lid set to 65 °C. The template amount and incubation time can be adjusted according to experimental needs.

4. Digest the DNA template: After transcription, add 2 μL of DNase I and incubate at 37 °C for 30 minutes. Stop the reaction by adding 1 μL of 500 mM EDTA (pH 8.0).

Note: After adding EDTA, proceed immediately with downstream RNA purification. Alternatively, DNase I digestion can be followed directly by purification without adding EDTA.

5. Purify the product.

6. Quantification and analysis of transcribed RNA:

(1) Measure RNA concentration using a UV spectrophotometer. If the RNA concentration is too high, dilute the sample before measurement.

(2) For RNA products of 100-1,000 nt, it is recommended to analyze on a 6% polyacrylamide/7 M urea denaturing gel using 1× TBE buffer.

10× TBE Buffer preparation: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA

Gel preparation (per 10 mL gel): 4.2 g urea, 4.4 mL RNase-free water, 1.5 mL 40% acrylamide (acrylamide:bis-acrylamide = 19:1), 1 mL 10× TBE buffer, 100 μL 10% AP, 10 μL TEMED. AP and TEMED should be added after urea is completely dissolved.

(3) For RNA products of 500-6,000 nt, it is recommended to analyze on a 1% formaldehyde agarose denaturing gel using 1× MOPS buffer.

10× MOPS Buffer preparation: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA

Gel preparation (per 100 mL gel): Weigh 1 g agarose, add to 72 mL RNase-free water, heat to dissolve. Add 10 mL 10× MOPS buffer. Cool solution to 50-60 °C, add 18 mL 37% formaldehyde, mix thoroughly, pour gel and allow to solidify.

(4) Electrophoresis: Take 0.2-1 μg RNA, dilute with RNase-free water to 5 μL, mix with an equal volume of 2× RNA Loading Buffer. Incubate at 70 °C for 10 minutes, then place on ice for 2 minutes before loading. After electrophoresis, stain the gel with GelStain or EB to visualize RNA. RNA markers should be treated in the same way as RNA samples (or follow the manufacturer's instructions).

- **Data Reference**

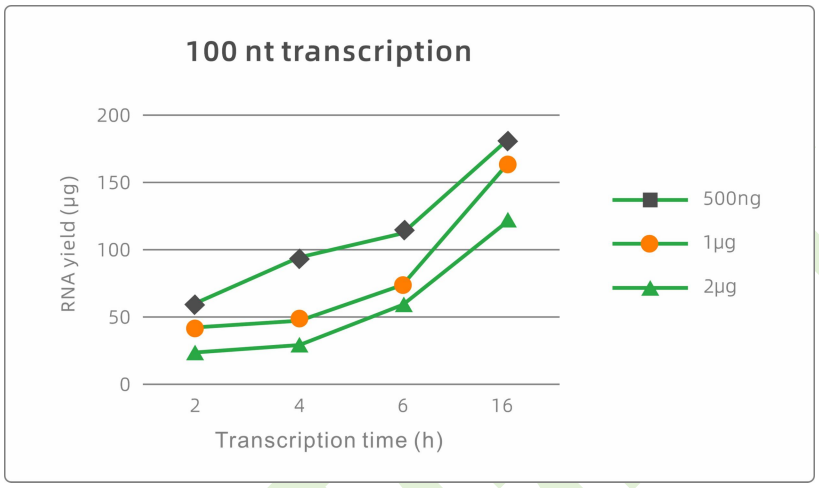

Figure 1. Relationship between RNA yield (for transcripts shorter than 300 nt) and template input as well as transcription time in a 20 μL reaction system