T7 Transcription Kit Instruction

Product Code

EDN-T7

Description

This kit is optimized for in vitro transcription using T7 RNA Polymerase. It efficiently transcribes DNA sequences downstream of a T7 promoter, using supercoiled plasmid DNA or linear DNA with a T7 promoter as a template. The kit is ideal for producing high-concentration RNA longer than 6000 nt. Using 1 μg of DNA template in a 20 μl reaction can yield 150–280 μg of RNA. For milligram-level RNA production, the reaction scale can be increased in parallel. The generated RNA is suitable for in vitro translation, RNase protection assays, RNA splicing, and hybridization probe labeling.

Storage

Store at -20°C for one year.

Component

Component	EDN-T701(25rxns)	EDN-T702(100 rxns)
T7 Transcription Enzyme Mix	50μl	200μl
5×T7Transcription 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 Volume	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

Operational Procedure

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

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: Pre-calculate the reaction system, and add the components in the following order: Water → Buffer → NTP → DNA Template → Enzyme.

3. Gently mix the components using a pipette and briefly centrifuge to collect them at the bottom of the tube. Incubate at 37°C for 2 hours.
Note: To prevent evaporation during prolonged transcription, it is recommended to conduct the reaction in a PCR machine with the heated lid set to 65°C. The amount of template and incubation time can be adjusted as needed.
4. DNA Template Digestion: After the reaction is complete, add 2 μl of DNase I and incubate at 37°C for 30 minutes. After digestion, add 1 μl of 500 mM EDTA (pH 8.0) to terminate the reaction (proceed to purification immediately after adding EDTA), or skip the EDTA and go directly to the purification steps.
5. Product Purification
6. Quantification and Detection of Transcription Products:
(1) Measure RNA concentration using a UV spectrophotometer. If the product concentration is extremely high, dilution prior to measurement is recommended.
(2) For RNA products ranging from 100 to 1000 nt, use a 6% polyacrylamide, 7M urea denaturing gel for detection, with 1× TBE Buffer as the electrophoresis buffer.

·**10× TBE Buffer**: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA.

· **Gel Preparation Method**: In every 10 ml, mix 4.2 g of urea, 4.4 ml of RNase-free water, 1.5 ml of 40% acrylamide (19:1 acrylamide), 1 ml of 10× TBE Buffer, 100 μl of 10% APS, and 10 μl of TEMED. Add APS and TEMED after the urea has completely dissolved.
(3) For RNA products ranging from 500 to 6000 nt, a 1% formaldehyde agarose denaturing gel is recommended for detection, with 1× MOPS Buffer as the electrophoresis buffer.

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

·**Gel Preparation Method**: In every 100 ml, weigh 1 g of agarose and add it to 72 ml of RNase-free water. Heat to dissolve, then add 10 ml of 10× MOPS Buffer. Once the solution cools to 50-60°C, add 18 ml of formaldehyde (37%), mix, and cast the gel.

(4) For electrophoresis detection, take 0.2–1 μg of RNA, dilute it to 5 μl with RNase-free water, and 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 the entire sample. After electrophoresis, stain with Gelstain or EB for visualization. Use the same treatment method for the RNA Marker as for the RNA samples (or refer to the supplier's instructions).

Reference Statistics

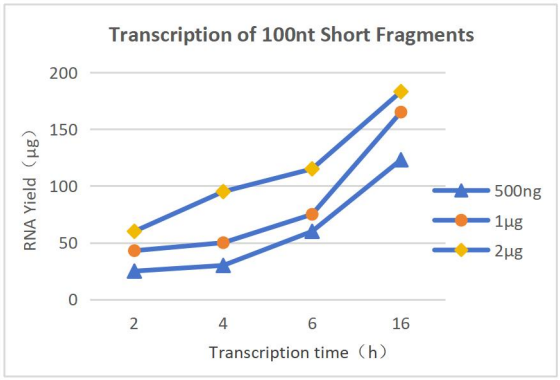

Figure 1. Relationship between RNA Transcription Yield and Template Input Amount/Transcription Time for RNA Lengths Less than 300 nt in a 20 μl Reaction System