T7 High Efficiency Transcription Kit

Cat. No. JT101

Storage: at -20°C for one year

Description

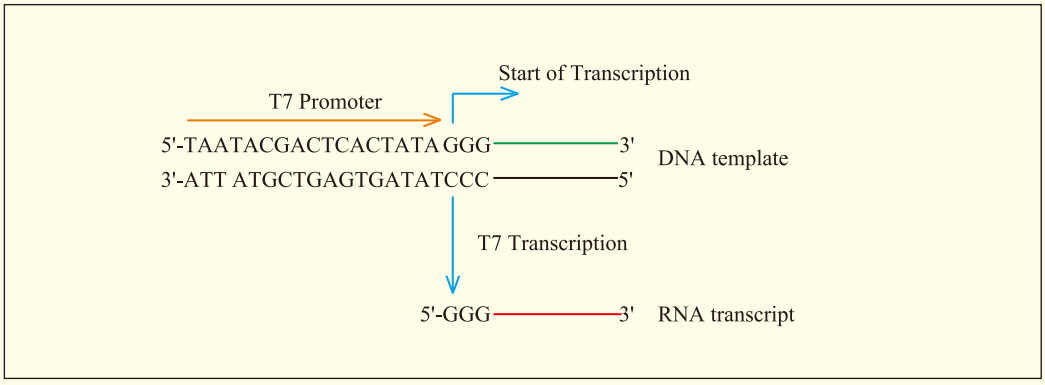
T7 High Efficiency Transcription Kit is designed for in vitro RNA synthesis by T7 RNA Polymerase with supercoiled or linearized DNA templates. More than 50 μg of RNA can be produced from a 20 μl reaction with 1 μg of template added. Synthesized RNA can be used for *in vitro* translation, RNase protection assays, RNA splicing, and hybridization assays.

Kit Contents

Component	**JT101-01 (25 rxns)**
T7 Transcription Enzyme Mix	50 μl
5×T7 Transcription Reaction Buffer	100 μl
10 mM NTP Mix	200 μl
DNase I (1 unit/μl)	25 μl
500 mM EDTA (pH 8.0)	25 μl
RNase-free Water	500 μl
Control Transcription Template (0.5 μg/μl)	10 μl

RNA Synthesis

Principle of *In Vitro* Transcription



Template Preparation

• Supercoiled plasmid DNA

Supercoiled plasmid DNA should contain a T7 promoter and an effective terminator. Termination efficiency varies with teminators. The following sequence has strong termination efficiency.

Transcription template

T7 Promoter ——————————— Terminator

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

Terminator: 5'-TTCCATCTGTTTTCTTATCTGTTCTTTCATCTGTTCTTTTATCTGTTTGTTT-3'

• Linearized DNA

Linearized plasmid DNA or PCR product, with T7 promoter and terminal sequences, can be used as template for *in vitro* transcription. We suggest to use 5'-overhang or blunt end restriction enzymes to generate the linearized templates, and avoid to use 3'-overhang restriction enzymes to generate the template. Digested linearized DNA should be purified.

Transcription

• Add following components

Component	Volume
Template	1 μg
5×T7 Transcription Reaction Buffer	4 μl
10 mM NTP Mix	8 μl
T7 Transcription Enzyme Mix	2 μl
RNase-free Water	to 20 μl

• Mix thoroughly and incubate at 37°C for 2 hours.

• Add 1 μl DNase I, incubate at 37°C for 15 minutes. Then add 1 μl of 500 mM EDTA (pH 8.0) to terminate reaction (immediately proceed to the following purification step after termination).

Purification of Synthesized RNA

Please refer to *EasyPure*® RNA Purification Kit

Quantification and Analysis of synthesized RNA

• RNA concentration can be determined by ultraviolet light spectrophotometer.

• Transcripts of 100-1000 nt can be run on denaturing gel (6% acrylamide, 7 M urea). Use 1×TBE Buffer as the running buffer. (10×TBE Buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA.)

• Transcripts of 500-5000 nt can also be run on 1% formaldehyde denaturing gel. Use 1×MOPS Buffer as the running buffer. (10×MOPS Buffer: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA.)

• For electrophoresis analysis, dilute 0.2-1 μg RNA with RNase-free water to make the total volume to 5 μl, add 5 μl of 2×RNA Loading Buffer and mix thoroughly, incubate at 70°C for 10 minutes and followed by incubation on ice for 2 minutes, then load samples on the gel. After electrophoresis, stain the gel.

Notes

• Avoid RNase contamination.

• Transcript produced from the control template is 640 nt.

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