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Datasheet

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T7 High Yield RNA Transcription Kit

Cat# EP5001 50 rxn
EP5002 100 rxn

Storage at -20˚C for one year

INTRODUCTION

T7 High Yield Transcription Kit is an optimized *in vitro* transcription reaction system using T7 RNA polymerase, and is suitable for *in vitro* production of large amount of RNA products, or to add the biotin or dye labelled nucleotides into RNA transcripts.

With this kit, 150-200 µg of RNA can be produced from 0.5 µg template in 20 µl reaction solution. The transcribed RNA can be used for various kinds of downstream applications, for example, research of structure or function of RNA, RNAi, microinjection, RNase protection assays, blots or *in situ* hybridization, and *in vitro* translation.

CONTENTS

No	Component	EP5001 - 50rxn	EP5002 - 100rxn
EA	T7 RNA Polymerase Mix	100 µl	200 µl
EB	10 × Reaction Buffer	100 µl	200 µl
EC	ATP Solution	100 µl	200 µl
ED	UTP Solution	100 µl	200 µl
EE	GTP Solution	100 µl	200 µl
EF	CTP Solution	100 µl	200 µl
EG	DNase I	50 µl	100 µl
EH	Control Template (0.5 µg/µl)	10 µl	20 µl
EI	RNase-free ddH_2O	1 ml	2 x 1 ml

PROTOCOL

Note: 1. DNA template should contain T7 RNA polymerase promoter at the upstream of the DNA template.
2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.

Additional Required Materials

DNA template: There should be right T7 RNA polymerase promoter at the upstream of the DNA template. The recommended concentration of template is 0.5 µg/µl, dissolved in water or TE buffer (10 mM Tris-HCl (pH 7-8), 1 mM EDTA).

RNase-free EP tubes and tips

37°C water bath or PCR machine

Necessary reagents and instruments for purification of RNA products

Preparation of DNA template

Different types of DNA containing T7 RNA polymerase promoter in its upstream sequence can be used as template, for example, linearized plasmid DNA, PCR products or synthesized DNA fragments. DNA template with concentration of 0.5 µg/µl in water or TE buffer (10 mM Tris-HCl (pH 7–8), 1 mM EDTA) is recommended for the reaction. Figure 1 shows the simplified structure of T7 RNA polymerase promoter, *in vitro* Transcriptional Start Site (TSS) and RNA transcript.

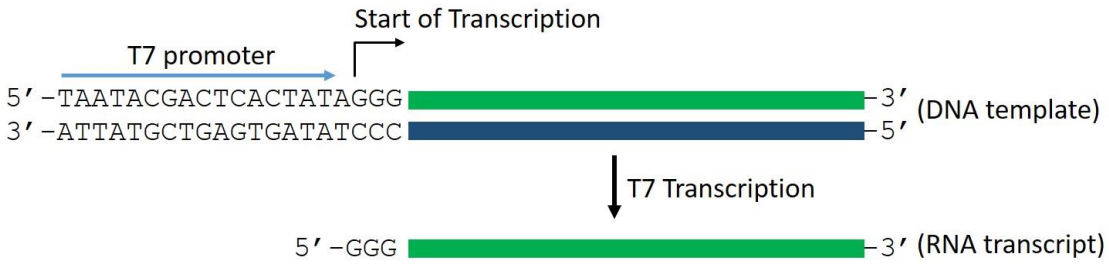

Figure 1. Transcription reaction catalyzed by T7 RNA polymerase

1. Plasmid as template

   The quality of plasmid, including its linearization and purity, will influence the yield and integrity of RNA transcript. Plasmid purified by different methods can be suitable as template, if there is no contamination of RNase, proteins, RNAs or salts.

   When use circular plasmid as template, different size of RNA transcript can be produced due to lack of efficient termination. To obtain specific length of RNA transcript, the plasmid needs to be completely linearized. Linearization can be produced by restriction enzyme digestion, and 5'-overhangs or blunt fragments are recommended. 3'- overhangs can generate spurious transcripts and should be avoid when do linearization. The linearized plasmid can be purified by column or phenol/chloride before *in vitro* transcription reaction. Please use 1 µl linearized plasmid for the 20 µl reaction solution.

2. PCR product as template

   PCR product with T7 promoter can be used as template for *in vitro* transcription. T7 promoter sequence (TAATACGACTCACTATAGGG) should be added at 5' end of upstream of the sequence to be transcribed. PCR product can be used for transcription without purification, but high yield will be obtained after purification. PCR product should be analyzed by agarose electrophoresis to make sure the specific amplicons before transcription reaction. For 20 µl reaction solution, the recommended amount of template is 0.1-0.5 µg of PCR product.

3. Synthesized DNA fragment as template

   Synthesized DNA fragment with T7 promotor can be used as template for *in vitro* transcription. For 20 µl reaction solution, the recommended amount of template is 0.1-0.5 µg of synthesized DNA fragments.

Protocol for *in vitro* transcription

Please wear gloves and use nuclease-free tubes and reagents during the operation to avoid the contamination of RNase.

1. Standard strategy
   1) Thaw all the required reagents and spin down briefly to collect reagents to bottom of the tube. Keep the reagents on ice before using.
   2) Prepare the reaction solution in order as below

Components	Volume
Nuclease-free Water	x µl
10 × Reaction Buffer	2 µl
ATP Solution	2 µl
GTP Solution	2 µl
UTP Solution	2 µl
CTP Solution	2 µl
DNA template (0.1-1 µg)	x µl
T7 RNA Polymerase Mix	2 µl
Total volume	20 µl

   3) Mix the reaction solution well and spin down briefly to collect the liquid. Incubate at 37°C for 2 hours.
   Attention: To avoid the influence of evaporation on reaction, it is recommended to use PCR machine to perform the reaction. As for synthesis of RNA transcript with small size (<0.3 kb), incubation time should be extended to 4 hours or more, to achieve the maximum yield. Overnight reaction (16 hours incubation) won't influence the quality of RNA transcript.
   4) Optional: Add 1 µl DNase I, and incubate at 37°C for 15 min to digest the DNA template.
   5) The synthesized RNA can be diluted and analyzed with agarose gel electrophoresis. Purified RNA products can be used for downstream reactions.
   Attention: Generally, one *in vitro* transcription reaction can produce a large amount of RNA transcript, and the concentration can be as high as 10 µg/µl. Please dilute the products with nuclease-free water before using.

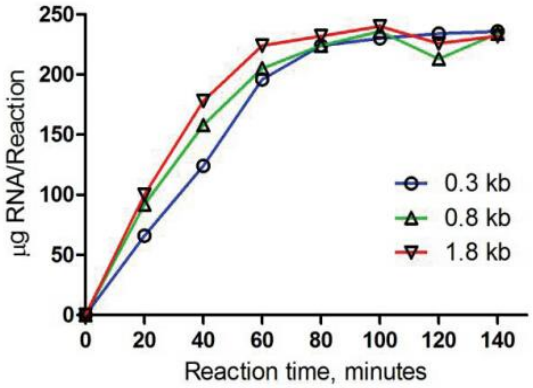

Figure 2. Accumulation curve of RNA transcript in standard reaction with 3 different size of templates.

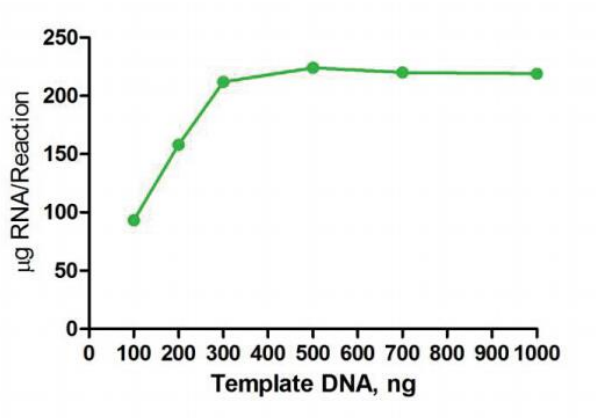

Figure 3. Accumulation of RNA transcript in strandard transcription reaction with different amount of 0.8 kb PCR product as templates after 2 hours incubation.

2. Strategy for adding modified nucleotide into RNA transcript

1) Thaw all the required reagents and spin down briefly to collect reagents to bottom of the tube. Keep the dissolved reagents on ice before using.

2) Prepare the reaction solution in order as below:

Components	Volume
Nuclease-free Water	x µl
10 × Reaction Buffer	2 µl
ATP Solution	2 µl
GTP Solution	2 µl
CTP Solution	2 µl
UTP Solution	1.5 µl
Modified UTP (10 mM)	5 µl
DNA template (0.1-1 µg)	x µl
T7 RNA Polymerase Mix	2 µl
Total volume	20 µl

3) Mix the reaction solution well and spin down briefly to collect the liquid. Incubate at 37°C for 2 hours.
Attention: To avoid the influence of evaporation on reaction, it's recommended to use PCR machine to perform the reaction. As for synthesis of RNA fragment with small size (<0.3 kb), incubation time should be extended to 4 hours or more, to achieve the maximum yield. Overnight reaction (16 hours incubation) won't influence the quality of RNA transcript. Modified nucleotide can decrease the efficiency of transcription, leading to lower yield than standard strategy.

4) Optional: Add 1 µl DNase I, and incubate at 37°C for 15 min to digest the DNA template.

5) The synthesized RNA transcript can be diluted and analyzed with agarose gel electrophoresis. Purified RNA products can be used for downstream reactions.
Attention: Generally, one *in vitro* transcription reaction can produce a large amount of RNA transcript, and the concentration can be as high as 10 µg/µl. Please dilute the products with nuclease-free water before using.

Purification of RNA transcript

Unmodified RNA transcript can be purified with phenol/chloride extraction or column. While for modified RNA products, we recommend to purify with column. If there is requirement for the length of the RNA transcript, please purify by gel extraction.

1. Phenol/Chloride purification

This method can remove protein and most of the free nucleotides of the products.

1) Add 160 µl of RNase-free water to dilute the reaction solution to final volume 180 µl. Mix well.
2) Add 20 µl of 3M sodium acetate (pH5.2) into the diluted reaction solution.
3) Use euqal volume of phenol/chloride (1:1, v/v) to extract RNA once. Use equal volume of chloride to extract RNA twice. Transfer the top layer (water phase) into a new EP tube.
4) Add 2 fold volume of ethanol into the supernant and mix well. Incubate at -20°C for at least 30 min. Centrifuge at 4°C for 15 min with maximum speed.
5) Discard the supernant, and add 500 µl of precold 70% ethanol to wash the RNA pellets.
6) Centrifuge at 4°C for 15 min with maximum speed. Discard the supernanta and dissolve the RNA pellet with RNase-free water or other buffer.
7) Store at -70°C.

2. Column purificaiton

With column purification, protein and free nucleiotides of the products can be removed.

1) Add 80 µl of RNase-free water to dilute the reaction solution to final volume 100 µl. Mix well.
2) Purify the RNA transcript according to the manual of column purification kit.
   Attention: Generally, 150-200 µg of RNA can be produced from every *in vitro* transcription reaction. More columns may be needed for binding of all the RNA transcript.

Quantification of RNA transcript

RNA transcript can be quantified by UV absorbance, but the free nucleotides affect the accuracy of the quantification. So, it is recommended to perform this method after purification.

We recommend to use RiboGreen® dye for RNA quantification. This method is not affected by free nucleotides and can be used to quantify RNA transcript before or after purification.

PRODUCT USE LIMITATION

These products are intended for research use only.