All In One High Efficiency mRNA Synthesis Kit Product Description

Overview

This kit adopts co-transcription and capping for IVT reaction, which is different from the traditional in vitro transcription of linear mRNA followed by 5' capping reaction. The cap analogue used in the reaction system of this kit can be recognized by T7 RNA polymerase, so that it can be automatically capped when transcription to achieve the purpose of one-step synthesis. This high-efficiency transcription kit that can generate 200 μg or more of mRNA in a 20 μL reaction system, with a capping rate generally above 95% and up to 99%.

Please note that this kit does not contain Poly(A) tailing system, Poly(A) tails need to be added additionally by other kits; or they can be designed after the DNA 3' UTR when constructing DNA transcription templates so tails can be added in one step during the IVT reaction without additional tailing. Please contact us for more support.

Components

Components	Concentration	Volume
H2O(RNase-free)	NA	1.5mL
ATP	100mM	75μL
CTP	100mM	75μL
UTP	100mM	75μL
m1Ψ	100mM	75μL
GTP	100mM	75μL

Buffer	10×	100μL
EnzymeMix	NA	450μL
DNaseI	NA	100μL
LiCl	NA	1.5mL

Operation process

1、DNA transcription template preparation

A good DNA transcription template is key to obtaining high quality mRNA. DNA template can be extracted by phenol chloroform isoamyl alcohol (phenol: chloroform: isoamyl alcohol = 25:24:1) and remove the protein including RNase, salt and other impurities.

Two methods of DNA template preparation: 1. PCR amplification, Poly(A) and other structures can be designed in the downstream primers, so as to form a large number of DNA linear templates with PolyA tails by PCR amplification; 2. plasmid fermentation amplification, i.e., the original plasmid is imported into Escherichia coli and other fermentation and amplification, and then purified and linearised by enzyme digestion.

Please pay attention to avoiding linearised DNA templates from producing 3′ prominent ends which will increase the production of spurious byproducts from the template in vitro. In addition, you need to ensure the DNA template is completely linearised (checked by agarose gel electrophoresis) to avoid the production of long-stranded RNA by-products.

2、Preparation of the IVT reaction system

Reagent preparation: EnzymeMix should always be placed on ice and other cool environments and mixed gently. NTP and cap analogues should be frozen at room temperature (there may be white precipitate after freezing,

which can be dissolved by mixing) and placed on ice in a cool environment. Reaction Buffer contains spermidine, which can form complexes with nucleic acids and precipitate as insoluble substances (especially in a freezing environment), so after freezing at room temperature, do not put it on ice during the preparation of the reaction system, but leave it in a room temperature environment. For all of the above reagents, centrifuge briefly after freezing to avoid loss and marginal contamination, and mix evenly before use.

Reaction system preparation: Add and prepare the IVT reaction system in the following order of precedence

IVT reaction system（20μL）	
ATP	1.5μL
m1Ψ or UTP (The kit provides N1-Methyl-Pseudouridine modified UTP, i.e., m1Ψ; and unmodified UTP, i.e., UTP; either of which can be selected, as required).	1.5μL
CTP	1.5μL
GTP	1.5μL
Cap analog (not included in this kit, must be purchased separately)	1.5μL
Mix gently	
Buffer	2.0μL
Mix gently	
DNA template	PCR template: 0.1μg - 0.2μg. Linearised plasmid template: 1μg. (This is the recommended amount, the actual demand can be designed to add different amounts to feel established)
EnzymeMix	9.0μL

Mix gently	
H_2O(RNase-free)	Total 20μL

The above system can be scaled up as needed.

3、Reaction

After mixing the above reaction system, briefly centrifuge and place the reaction in a thermostatic reactor (or PCR machine) at 37℃ for 2 ~ 4 h. It is recommended that the reaction be performed for 4 h, and the yield is generally higher than 2 h. Adjust the reaction time according to the target length and the desired RNA yield, which is usually no more than 16 h. The reaction time required for maximum yield varies for different DNA sequence templates.

At the end of the reaction, a white precipitate may be produced, which is magnesium pyrophosphate produced by the pyrophosphate released by the reaction and the magnesium ions in the reaction solution, and will not affect the subsequent operation.

4、Termination of the reaction

DNaseI was briefly centrifuged and placed in a cool environment such as on ice, mixed and used.2 μL of DNaseI was added to 20 μL of the reaction system to digest the DNA template, and the reaction was carried out at 37℃ for 15 min.

5、Purification Recycling

Commercial purification kits can be used to recycle the above mRNA. LiCl precipitation is also a commonly used method for mRNA recycle. Methods as follows:

(1) 22 μL IVT termination reaction solution + 30 μL RNase-free H_2O + 30 μL LiCl solution (melt at room temperature, mix well, and place on ice and other cooling environment), mix completely, and then precipitate for 1h at -20℃;

(2) Centrifuged at 16000g for 15min at 4℃, white precipitation of mRNA

was visible at the bottom of the tube, carefully remove the supernatant as much as possible;

(3) Add 1mL of pre-cooled 70% ethanol on ice to wash the white precipitate of mRNA and the wall of the tube (rotate to wash, do not blow suction with a gun tip);

(4) Centrifuge at 16000rpm, 4℃ for 5min, carefully remove the supernatant as much as possible;

(5) Let the residual ethanol dry for 5~10min on the ultra-clean bench, then add the appropriate amount of RNase-free H2O or other RNA buffer and let it dissolve for 5~20min, mix gently and store it at -20℃~-80℃.

6、mRNA quantification and detection

mRNA can be quantified by instruments such as NanoDrop or conventional nucleic acid quantification methods.The purity and integrity of mRNA bands can be detected by capillary electrophoresis or agarose gel electrophoresis as follows:

Take 400ng of mRNA product, add 2 x RNA Loading (Sangon, No. B548640-0001; or DNA loading buffer can be used) 1:1, mix well and put it into PCR instrument at 70℃ for 5min, take the denatured sample and put it into 1% agarose electrophoresis for detection at 130V for 20min (the specific electrophoresis conditions can be adjusted according to the actual situation).

7、Precautions for operation

If the DNA template, reagents, tubes, micropipette tips or other materials used in the reaction are mixed with DNase/RNase contamination, it will lead to the decrease of mRNA yield and degradation of the mRNA obtained by using this kit. Please use special DNase/RNase-free sterilised tubes and micropipette tips in the reaction and wear new disposable gloves to prevent DNase/RNase contamination.

References

1. Mu X, Greenwald E, Ahmad S, et al. An origin of the immunogenicity of in vitro transcribed RNA[J]. Nucleic acids research, 2018, 46(10): 5239-5249.
2. Vaidyanathan S, Azizian K T, Haque A K M A, et al. Uridine depletion and chemical modification increase Cas9 mRNA activity and reduce immunogenicity without HPLC purification[J]. Molecular Therapy-Nucleic Acids, 2018, 12: 530-542.
3. Schenborn E T, Mierendorf Jr R C. A novel transcription property of SP6 and T7 RNA polymerases: dependence on template structure[J]. Nucleic acids research, 1985, 13(17): 6223-6236.