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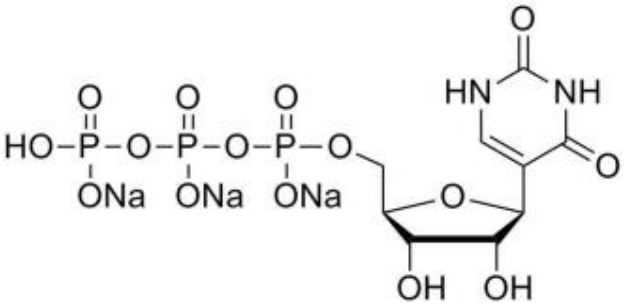
Pseudo UTP 100mM

Product Number: M062003

Shipping and Storage

This product is stored and transported below -20℃, and to avoid repeated freezing and thawing.

Description

Product name	Pseudo UTP 100mM
Structure	
Concentration	100 mM
Product Number	M062003
Specification	1，20，100，500 mL

Pseudo UTP 100mM is a 100mM colorless aqueous solution, titrated with NaOH to pH 6.9-7.1, with a purity greater than 99% (HPLC) and high stability. It has been tested for in vitro transcription function. This product can be used for various molecular biological reactions such as aRNA synthesis, siRNA synthesis, RNA amplification, and in vitro transcription.

Protocol

1. It is necessary to use sterile (DNase/RNase-Free) reagents, pipette tips, reaction tubes and reagent bottles, etc., to prevent RNase contamination during operation, and the surface of the operating table and pipette can be cleaned with RNase agent wipe.
2. The product should be stored in the ice box or ice bath after dissolution, and should be stored below -20℃ immediately after use.
3. RNA polymerase: In vitro transcription usually uses monosubunit phage polymerases derived from phages T7, T3, SP6, K1-5, K1E, K1F, or K11, with T7 RNA polymerase being the most commonly used.
4. DNA template: linearized plasmids, PCR products or synthetic DNA oligonucleotides can be used as templates for in vitro transcription, and the upstream of the sequence to be transcribed contains promoter regions corresponding to RNA polymerase (such as T7 promoter, SP6 promoter, etc.).
5. Reagents required for the reaction
   - 5.1. DNA Template
   - 5.2. Nucleoside triphosphates (NTP) and modified nucleotide phosphates (N1-Me-pUTP, pUTP)
   - 5.3. T7 RNA polymerase
   - 5.4. RNase inhibitor
   - 5.5. DNase/RNase-Free Water
6. Reaction system and conditions
   - 6.1. The components except T7 RNA polymerase and RNase inhibitor were shaken and mixed, centrifuged briefly, collected at the bottom of the tube, and stored on ice for future use. Add the components at room temperature in the following order[1]:

Reagent	Volume	Final Conc.

DNase/RNase-Free Water	Add to a total volume of 20μL	—
10×The reaction buffer	2μL	1×
ATP (100 mM)	2μL	10mM
CTP (100 mM)	2μL	10mM
GTP (100 mM)	2μL	10mM
UTP[2](100 mM)	2μL	10mM
RNA enzyme inhibitor(40units /μL)	1μL	2units /μL
T7 RNA Polymerase(50 units /μL)	2μL	5units /μL
DNA templet	Either 1 or 0.5μg[3]	And 50 or 25μg / mL[3]
Bulk volume[4]	20μL	——

Note:1)The high concentration of salt ions in the reaction buffer will affect the enzyme, and the high concentration of spermidine will precipitate the DNA template, so water, reaction buffer and NTP should be added first, and the enzyme and template should be added after gently mixing with a pipette.

2)Modified nucleoside triphosphates (N1-Me-pUTP, pUTP) can be substituted for wild-type UTP at the same concentration as wild-type UTP.

3)The final concentration of plasmid template DNA was 50μg/mL, and the final concentration of PCR product as template was 25μg/mL.

4)The reaction system can be expanded according to the equal proportion.

6.2. Gently mix each component with a pipette, centrifuge briefly, and incubate at 37℃ for 2-3hours. If the transcription length is less than 300 nt, the reaction can be extended to 4-8 h. In orderto avoid the effect of evaporation on the reaction system, it is recommended to perform the reaction in a PCR instrument.

6.3. After the reaction, DNase I (1unit) was added and incubated at 37℃ for 15 min to remove theDNA template (optional). Compared to the product RNA, the template DNA is very low in contentand can generally be digested with DNase I without removal.