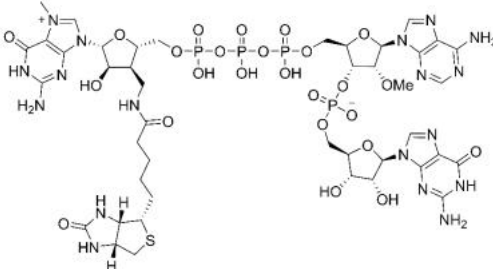


LZCap™ AG(3'Ma-Biotin)

Product description

LZCap™ AG(3'Ma-Biotin) is a Cap 1 analog with a Biotin label, which can be used as the capping agent for producing mRNAs in an "one-pot" process. Through T7 polymerase, mRNA with 5' end Cap 1 structure was generated by co-transcription using LZCap™ AG(3'Ma-Biotin), NTPs, and template DNA. The capped mRNA could be directly translated and expressed in cells and in vivo. mRNA capped with LZCap™ AG(3'Ma-Biotin) can be bound to streptavidin magnetic beads for biological functional exploration. Moreover, Biotin, as a common molecule in the body, is involved in various intracellular biological functions and exhibits strong binding affinity with many proteins. Biotin-labeled mRNA holds potential for exploration in biology.

Specifications

Cat.No.	10691ES10 / 10691ES60
Size	50 µL / 100 µL
Molecular Formula	C ₄₃ H ₆₀ N ₁₈ O ₂₅ P ₄ S (Free acid)
Molecular Weight	1385.01 (Free acid)
Concentration	25 mM
Purity	HPLC ≥90%
Salt type	NH ₄ ⁺
Structure	

Components

Name	10691ES10	10691ES60
LZCap™ AG(3'Ma-Biotin)	50 µL	100 µL

Storage

This product should be stored at -25~-15°C, Valid for 2 years.

LZCap™ DNA Template Design

LZCap™ AG(3'Ma-Biotin) is suitable for AG-initiated sequences. As shown in the figure below, the T7 promoter (underlined) followed by the AG sequence can effectively initiate transcription.

5'TAATACGACTCACTATA **AG** GNNNNNNNNNNNNNNNNNNNNNN 3'
3' ATTATGCTGAGTGATAT **TC** CNNNNNNNNNNNNNNNNNNNNNN 5'

↓ T7 polymerase transcription+ LZCap™ AG
5' **GAG**GNNNNNNNNNNNNNNNNNNNNNN 3'

Protocol

1. Thaw components required for the experiment on ice.
2. Add RNase free water and NTPs to reaction tube. Then add LZCap™ AG(3'Ma-Biotin) Cap analog to tube and vortex briefly to collect liquid.
3. Add 10X Transcription Buffer. Vortex. Spin briefly to collect Liquid. Then add DNA template.
4. Add Murine RNase Inhibitor, Yeast Inorganic Pyrophosphatase, and T7 RNA Polymerase.
5. Mix well by flicking or inverting tube 10 times and spin briefly to collect liquid.
6. Incubate at 37 °C for 2-3 hours. If the transcript length is less than 100 nt, increase the reaction time to 4-8 h.

Component	Volume (μL)	Final concentration
RNase Free Water	Up to 20 μL	/
ATP(100 mM)	1	5 mM
UTP(100 mM)	1	5 mM
CTP(100 mM)	1	5 mM
GTP(100 mM)	1	5 mM
LZCap™ AG(3'Ma-Biotin) (25 mM)	3.2	4 mM
10×Transcription Buffer	2	1×
Linear DNA	1 μg	50 ng/μL
Recombinant RNase Inhibitor(40 U/μL)	0.5	1 U/μL
Pyrophosphatase(0.1 U/μL)	0.4	0.002 U/μL
T7 RNA polymerase(250 U/μL)	0.64	8 U/μL
Final Volume	20 μL	

Notes

1. LZCap™ AG(3'Ma-Biotin) is suitable for T7 promoter transcription vector with 5 'AG 3' initiated sequences, which needs to be considered when constructing the vector.
2. The reagents, consumables and containers used in the experiment are free of RNase contamination.
3. It is recommended to use a linearized DNA template for transcription.
4. When modified nucleotides were used in place of wild-type nucleotides, the final concentration of the reaction was unchanged.
5. Modified N1-Me-pUTP can be used in place of wild-type UTP. The modified N1-Me-pUTP reduces the immunogenicity of mRNA. Henovcom can also provide modified nucleotide N1-Me-pUTP (Cat. No.: HN1002).
6. If the PCR product is used as the transcription initiation DNA template, the amount of DNA template can be reduced by half.