Version 24.10

## LZCap®AG(3'Ma-Biotin)

**Description:** LZCap®AG(3'Ma-Biotin) is a Cap1 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.

**Molecular Formula:**  $C_{43}H_{60}N_{18}O_{25}P_4S$  (Free acid)

Molecular Weight: 1385.01 (Free acid)

CAS No.: /

Concentration: 25 mM

Specifications: 50 µL, 100 µL

Purity: HPLC ≥90%

Salt type: NH<sub>4</sub><sup>+</sup>

Structure:

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Storage Conditions: store at -15°C or below.



## 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 GNNNNNNNNNNNNNNNNNNNNN 3'
- 3' ATTATGCTGAGTGATAT TC CNNNNNNNNNNNNNNNNNNNNNN 5'

T7 polymerase transcription+ LZCap®AG

5' GAGGNNNNNNNNNNNNNNNNNNNNNN 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 100nt, increase the reaction time to 4-8 h.

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



## 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.