Version 24.10

LZCap®AG(3'Ma-Cy5)

Description: LZCap®AG(3'Ma-Cy5) is a Cap1 analog with a Cy5 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-Cy5)，NTPs，and template DNA. The capped mRNA could be directly translated and expressed in cells and in vivo. For detecting the Cy5 fluorescence of LZCap®AG(3'Ma-Cy5) mRNA, the recommended wavelengths are (640/675).

Molecular Formula： $C_{66}H_{84}N_{18}O_{30}P_4S$ (Free acid)

Molecular Weight： 1797.51 (Free acid)

CAS No.： /

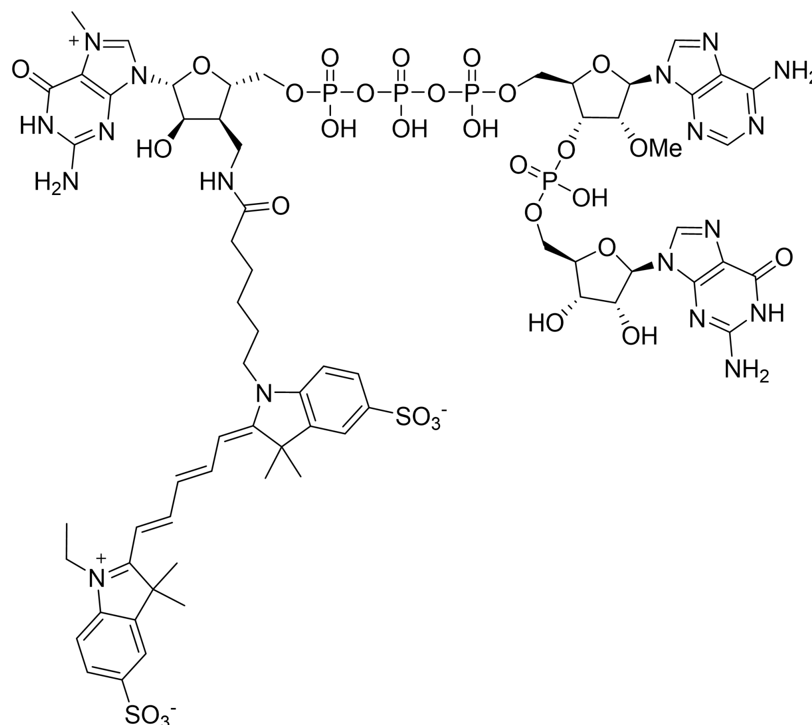
Concentration： 25 mM

Specifications： 50 μL、100 μL

Purity：HPLC ≥90%

Salt type： NH_4^+

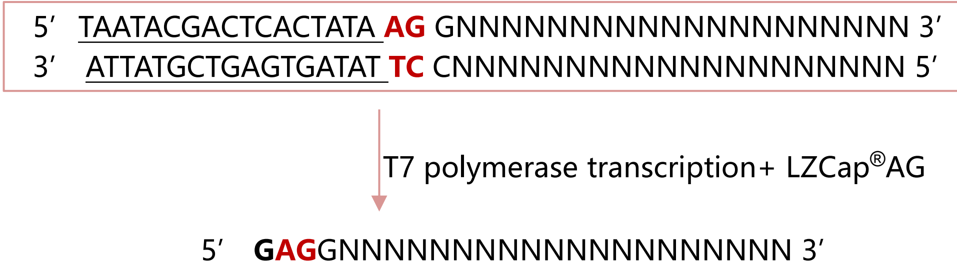
Structure：



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

LZCap® DNA Template Design

LZCap®AG(3'Ma-Cy5) 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.



Protocol

1. Thaw components required for the experiment on ice.
2. Add RNase free water and NTPs to reaction tube. Then add LZCap®AG(FM) Cap analog to tube and vortex briefly to collect liquid.
3. Add 10x m6A 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-Cy5) (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-Cy5) is suitable for T7 promoter transcription vector with 5 ‘AG 3’ initiated sequences, which needs to be considered when constructing the vector.
2) LZCap®AG(3'Ma-Cy5) and its mRNA products should be stored and used away from light.
3) The reagents, consumables and containers used in the experiment are free of RNase contamination.
4) It is recommended to use a linearized DNA template for transcription.
5) When modified nucleotides were used in place of wild-type nucleotides, the final concentration of the reaction was unchanged.
6) 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).
7) If the PCR product is used as the transcription initiation DNA template, the amount of DNA template can be reduced by half.
8) During the purification of post-transcriptional mRNA, it is enough to wash the mRNA precipitate once with pre-cooled 75% ethanol. Washing it repeatedly for many times will affect the fluorophore.