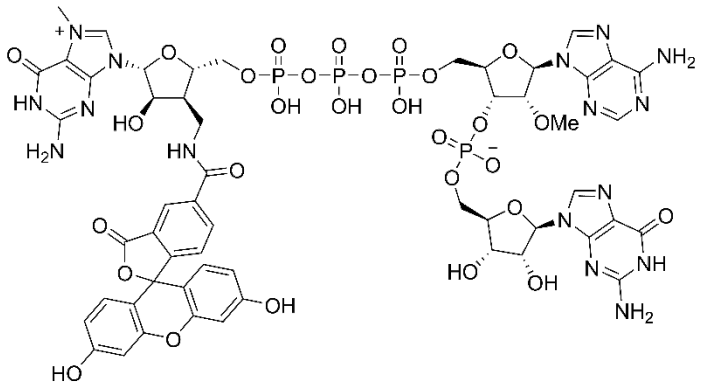
Version 23.10

LZCap®AG(3'Ma-FAM)

Description: LZCap®AG(3'Ma-FAM) is a Cap1 analog with a FAM label, can be used as the capping agent for producing mRNAs in an "one-pot" process. Under the action of T7 polymerase, mRNA with 5' end Cap 1 structure was generated by co-transcription using LZCap®AG(3'Ma-FAM), 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-FAM) can be detected for FAM fluorescence using flow cytometry and fluorescence microscopy, enabling tracking and localization of mRNA and LNP distributions.

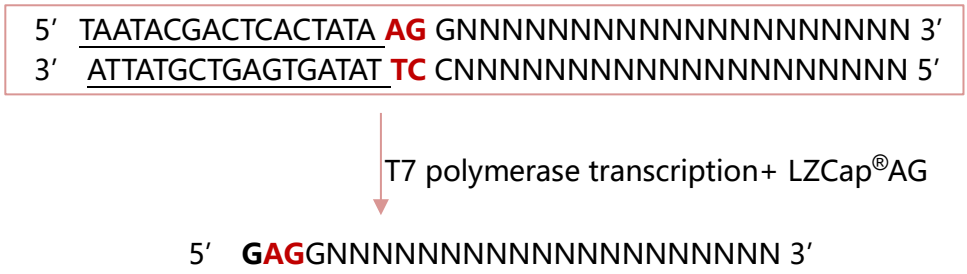
Molecular Formula: $C_{54}H_{56}N_{16}O_{29}P_4$ (Free acid)
Molecular Weight: 1517.02 (Free acid)
CAS No.: /
Concentration: 25 mM
Specifications: 50 μL、100 μL
Purity: HPLC ≥90%
Salt type: NH_4^+
Structure:



Storage Conditions: store at -20°C or below.

LZCap® DNA Template Design

LZCap®AG(3'Ma-FAM) 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. Refer to the following reaction system to configure the transcription system at room temperature.

Component	Volume (μL)	Final concentration
ATP(100mM)	1	5mM
UTP(100mM)	1	5mM
CTP(100mM)	1	5mM
GTP(100mM)	1	5mM
LZCap®AG(3'Ma-FAM) (25mM)	3.2	4mM
10×Transcription Buffer	2	1×
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
Linear DNA+RNase Free Water	9.26	1μg
final volume	20μL	

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).

Notes:

1) LZCap®AG(3'Ma-FAM) 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) If the PCR product is used as the transcription initiation DNA template, the amount of DNA template can be reduced by half.

3. Mix the prepared reaction solution, centrifuge briefly, and incubate at 37°C for 2-3 hours. If the transcript length is less than 100nt, increase the reaction time to 4-8 h.