Version 24.11

LZCap®AG(3'Ma-Cy5) Capping Kit (N1-Me-pUTP)

Description: LZCap®AG(3'Ma-Cy5) Capping Kit is the co-transcriptional synthesis of mRNA containing the Cap1 structure *in vitro*. The core component LZCap®AG(3'Ma-Cy5) is a Cap1 analog that can be added to the 5' end of mRNA in the presence of T7 polymerase. DNase I and LiCl are included in the kit for mRNA purification. Recommended wavelengths for detecting the Cy5 fluorescence of LZCap®AG(3'Ma-Cy5) mRNA: (640/675).

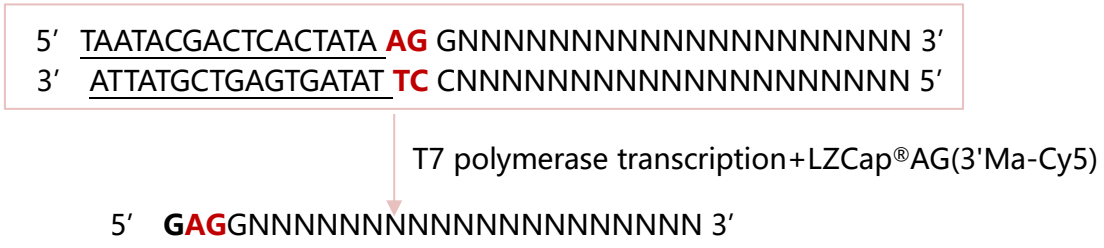
Specifications and Components: 20T/Kit (20μL)

No.	Component	Concentration	20 Test
1	LZCap®AG(3'Ma-Cy5)	25 mM	64 μL
2	ATP	100 mM	20 μL
3	N1-Me-pUTP	100 mM	20 μL
4	CTP	100 mM	20 μL
5	GTP	100 mM	20 μL
6	10×Fluro Transcription Buffer	/	40 μL
7	Enzyme Mix	/	80 μL
8	Recombinant DNase I(RNase-free)	5 U/μL	40 μL
9	LiCl	7.5 M	1 mL
10	Control	0.5 μg/μL	4 μL
11	RNase Free Water	/	2 mL

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

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.

Component	Volume (μL)	Final concentration
ATP (100 mM)	1μL	5 mM
N1-Me-pUTP (100 mM)	1μL	5 mM
CTP (100 mM)	1μL	5 mM
GTP (100 mM)	1μL	5 mM
LZCap®AG(3'Ma-Cy5) (25 mM)	3.2μL	4 mM
10×Fluro Transcription Buffer	2μL	1×
Enzyme Mix	4μL	/
Linear DNA+ RNase Free Water	6.8μL	50 ng/μL
终体积	20μL	

2. Mix the prepared reaction solution, centrifuge briefly, and incubate at 37°C for 2-3 hours. The reaction time should be increased to 4-8 h when the length of transcript is less than 100 nt.
3. After the reaction, 2μL of DNase I is added to each tube and the DNA template will be digested at 37° C for 15min.
4. Purification of mRNA by LiCl precipitation
   1) Add 50μL of LiCl and 30μL of RNase Free Water to 10μL of transcript mRNA after the reaction (the final concentration of LiCl should be kept at 4.5-4.8 M), mix well and incubate at -20℃ for at least 0.5h.
   2) Centrifuge the mixture at 12000rpm for 15min, and remove the supernatant and preserve the precipitate.
   3) Wash the precipitate with 600μL of pre-chilled 75% ethanol, centrifuge at 12000 rpm for 8 min, and remove the supernatant.
   4) The purified mRNA should be dried for 10 min until the ethanol evaporated completely and is re-dissolved with 30-100μL RNase-Free water.

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 this experiment are free of RNase and DNase contamination.
4) It is recommended to use a linearized DNA template for transcription.

5) When modified nucleotides is used in place of wild-type nucleotides, the final concentration of the transcript is not affected, but the UV ratio of 260/280 may be different from regular RNA.
6) If the PCR product is used as the DNA template, the amount of DNA template can be reduced by half.
7) Due to the high concentration of 10×Fluro Transcription Buffer, the high salt environment will lead to polymerase inactivation. When preparing the reaction solution, we need to add water first, then buffer, NTPs and LZCap®AG(3'Ma-Cy5), DNA template, and finally the enzymes.