Ver. HB221110

mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μl)

Product description

mRNA Cap 2´-O-Methyltransferase is a recombinant protein derived from vaccinia virus. The enzyme can add a methyl group at the 2´-O position of the first nucleotide of the cap structure at the 5´ end of the RNA. The enzyme uses SAM as a methyl donor to methylate capped RNA to form a Cap1 structure. The Cap1 structure can enhance the translation efficiency of mRNA, so it can improve the expression of mRNA in transfection and microinjection experiments. This enzyme requires RNA with m7GpppN, a 7-methylguanosine cap structure, as a substrate.

This product is produced in accordance with GMP process requirements. The product is provided in liquid form and can be used for Cap1 capping reaction of pre-mRNA in vivo/in vitro.

Components

Components No.	Name	10612ES92 (10,000 U)	10612ES97 (50,000 U)	10612ES98 (250,000 U)	10612ES99 (20 MU)
10612	mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μl)	200 μL	1 mL	5 mL	400 mL

Specifications

Source	Recombinant *E.coli* with Cap 2´-O-Methyltransferase gene
Optimum Temperature	37°C
Storage Buffer	20 mM Tris-HCl pH8.0，0.1mM EDTA，1mM DTT，100mM NaCl，50%（v/v）glycerin，0.1%（v/v）Trion X-100
Unit Definition	One unit is defined as the amount of enzyme required to methylate 10 pmol of 80nt capped RNA transcript in 1 h at 37°C.

Shipping and Storage

The mRNA Cap 2´-O-Methyltransferase GMP-grade products are shipped with dry ice and can be stored at -15°C ~ -25°C for one year.

Instructions

1. **Cap1 capping reaction** (20 μL reaction system)

This step is suitable for capping reaction of 10 μg RNA (≥ 100 nt), and can be amplified according to experimental needs.

1) Take 10 μg RNA to a 1.5 mL centrifuge tube and dilute to 9.5 μL with nuclease-free water.

2) Heat at 65°C for 5 minutes.

3) Take out the centrifuge tube and place it on ice for 5 minutes.

4) Add the following components in sequence:

Components	Volume

Denatured RNA	9.5 μL
10×Capping Buffer	2.0 μL
Murine RNase inhibitor (40U U/μL)	0.5 μL
GTP (10 mM)	1.0 μL
SAM (10 mM, fresh)	1.0 μL
Vaccinia Capping Enzyme (10 U/μL)	5.0 μL
Cap 2´-O-Methyltransferase (50 U/μl)	1.0 μL

【Note】10×Capping Buffer(Cat# 10666)：0.5 M Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT pH 8.0 at 25°C.

5）Incubate at 37°C for 2 hours.

6）RNA capping is completed, next experiments can be performed.

Notes

1. For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.

2. The extracted RNA needs to be purified and resuspended in nuclease-free water.

3. The RNA solution needs to be heated before adding the enzyme to remove the secondary structure at the 5' end.

4. For RNA with a known 5'end structure, the reaction time can be extended to 4 hours to improve the capping efficiency.

5. In the 5'end labeling reaction system, the GTP stock solution should be diluted to 1-3 times of the mRNA molar concentration in the reaction system.