HB220701

mRNA Vaccinia Capping Enzyme GMP-grade (10 U/μL)

Product Information

Product Name	Catalog No.	Size
mRNA Vaccinia Capping Enzyme GMP-grade (10 U/μL)	10614ES84	2 KU
	10614ES92	10 KU
	10614ES96	100 KU
	10614ES99	5 MU

Product Description

Eukaryotes mRNA forms a special structure at the 5'end after transcription, which is the cap structure. The cap structure plays an important role in the stability, transportation and translation of mRNA. Vaccinia virus capping enzyme is an effective enzyme that can catalyze the formation of the cap structure. It's composed of two subunits D1 and D12. It also has RNA triphosphatase activity, guanylate acyltransferase activity and guanine methyltransferase activity, could connect the 7-methylguanine cap structure (m7Gppp) to the 5'end of the RNA (m7Gppp5'N). Vaccinia virus capping enzyme can cap the RNA at correct direction within one hour when present at suitable concentration of capping buffer, guanosine triphosphate (GTP), S-adenosylmethionine (SAM), etc..

This product is produced in accordance with GMP process requirements and provided in a liquid form, used for in vivo/in vitro pre-translation mRNA capping reaction or mRNA 5'end labeling reaction.

Product Properties

Source	Recombinant *E. coli* with vaccinia virus capping enzyme gene
Optimum Temperature	37℃
Storage Buffer	20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% glycerin
Unit Definition	1 unit: The amount of enzyme required to incorporate 10 pmol GTP (α-^{32}P) into a transcript with 80 nucleotides (80 nt) at 37℃ within 1 h.

Contents

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		10614ES84 (2 KU)	10614ES92 (10 KU)	10614ES96 (100 KU)	10614ES99 (5 MU)
10614	mRNA Vaccinia Capping Enzyme GMP-grade (10 U/μL)	200 μL	1 mL	10 mL	500 mL

Shipping and Storage

mRNA Vaccinia Capping Enzyme GMP-grade products are shipped with dry ice and can be stored at -15℃ ~ -25℃ for one year.

Experimental methods

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℃ for 5 min;

3. Take out the centrifuge tube and place it on ice for 5 min;

4. Add the following components in sequence:

Components	Volume
Denatured RNA	9.5 μL
10×Capping Buffer	2.0 μL
Murine RNase inhibitor(40 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 @ 25°C.

5. Incubate at 37°C for 2 h;

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 h 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.