

Product Description

The mRNA Capping Kit independently developed by Biori Biotech contains Vaccinia Capping Enzyme (VCE) and mRNA Cap 2'-O-Methyltransferase (2OM) internally. Vaccinia Capping Enzyme efficiently adds an m7G cap to the 5' end of mRNA, thereby producing RNA with a Cap0 structure. 2'-O-Methyltransferase adds a methyl group to the 2'-O position of the first nucleotide following the cap structure of Cap0 RNA, forming RNA with a Cap1 structure.

This kit enables simultaneous capping by VCE and 2OM in a single-tube reaction, directly yielding Cap1 RNA. It can significantly enhance the intracellular stability of mRNA and promote translation efficiency.

Components

Components	Cat. No.	Volume
Vaccinia Capping Enzyme	BP-E05	50 μ L
2'-O-Methyltransferase	BP-E06	50 μ L
10 \times Capping Buffer	BP-AS-12	100 μ L
GTP Solution (10 mM)	BP-AS-02	50 μ L
SAM (32 mM)	BP-AS-06	25 μ L
RNase Inhibitor (40 U/ μ L)	BP-E02	50 μ L
RNase-free ddH ₂ O	BP-AS-11	1 mL

Storage

Store at $-20\pm 5^{\circ}\text{C}$.

Protocol

1. mRNA Preparation

1.1 Prepare a sufficient quantity of mRNA. After recovery, resuspend it in RNase-free ddH₂O and measure the concentration.

1.2 Recommended dosage: Use 20 μ L system for capping per 50 pmol of RNA.

1.3 Rough calculation of 50 pmol RNA content: RNA amount (ng) = 0.05 nmol \times 330 g/mol \times X nt (X = number of RNA nucleotides).

2. Capping Reaction

2.1 Take out SAM (32 mM) and thaw it on ice. Dilute it to 4 mM before adding it to the reaction mixture. GTP (10 mM) and 10 \times Capping Buffer can be thawed at room temperature. Since the methyl donor SAM is a high-energy methyl donor and inherently unstable, it is recommended to perform all pipetting steps at low temperature.

2.2 Prior to the capping reaction, it is recommended to thermally denature the RNA by heating at 65 $^{\circ}\text{C}$ for 5 minutes, then immediately placing it on ice for 5 minutes.

2.3 Add each component sequentially according to the table below:

Components	Volume
10 \times Capping Buffer	2 μ L
GTP Solution(10 mM)	1 μ L
SAM (4 mM)	1 μ L
Vaccinia Capping Enzyme	1 μ L
2'-O-Methyltransferase	1 μ L
RNase Inhibitor (40 U/ μ L)	0.5 μ L
Denatured RNA	50 pmol
RNase-free ddH ₂ O	Up to 20 μ L

Incubation at 37 $^{\circ}\text{C}$ for 1 hour is sufficient to complete capping. If capping more RNA with an equivalent amount of enzyme is required, the incubation time at 37 $^{\circ}\text{C}$ can be appropriately extended to 2 hours.

3. Concentration and Activity Definition: 10U/ μ L

3.1 One unit of VCE is defined as the amount of enzyme required to incorporate 10 pmol of GTP into RNA in 1 hour at 37 $^{\circ}\text{C}$.

3.2 One unit of 2OM is defined as the amount of enzyme required to incorporate 10 pmol of methyl groups into Cap0 RNA

in 1 hour at 37°C.

Notes

1. The capping reaction is highly sensitive to RNase. It is crucial to strictly avoid RNase contamination in the reaction system. All experimental equipment, such as pipette tips and microcentrifuge tubes, must be RNase-free. The system should be prepared in an RNase-free environment whenever possible; a cleaned and certified clean bench can be used for this operation. Furthermore, synthesized RNA should be opened and handled within the clean bench area to avoid RNase contamination.
2. The efficiency of this capping reaction is influenced by the structure of the RNA 5' end. Therefore, it is recommended to denature the RNA by heating (65°C for 5 min, followed by immediate placement on ice for 5 min) to disrupt any higher-order structures at the 5' end.
3. This capping reaction is typically completed within 1 hour. If the RNA 5' end structure is complex or the RNA is relatively short (≤ 200 nt), the reaction time can be extended to 2 hours.
4. Mix thoroughly before use and avoid repeated freeze-thaw cycles.