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INSTRUCTIONS FOR USE

Product Name: mRNA Vaccinia Capping Enzyme, GMP-Grade (10 U/μL) **Catalog # VAC01-E59B**

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.

Step 1. In a 1.5 ml centrifuge tube add 10 μg of RNA and dilute to 9.5 μL with nuclease-free water.

Step 2. Heat for 5 minutes at 65°C.

Step 3. Place the centrifuge tube on ice for 5 minutes.

Step 4. Add the following components in sequence:

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

Note: lOx Capping Buffer: 0.5 M Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT pH 8.0 at 25°C.

Step 5. Incubate at 37°C for 2 hours.

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