

Vaccinia Capping Enzyme

v. 240101

Catalog number	C15037-500U	
Package & Component	Name	Amount
	Vaccinia Capping Enzyme (10,000 U/mL)	500 U (1 vial)
	10X Capping Enzyme Reaction Buffer	100 μ L (1 vial)
Description	This capping enzyme contains three enzymatic activities (RNA triphosphatase, guanylyltransferase and guanine methyltransferase) to forming a Cap 0 structure (m ⁷ Gppp5'N) In the presence of the vaccinia capping enzyme, GTP and SAM (methyl donor) the In Vitro Transcripts mRNA can be efficient add capped in less one hour.	
Source	<i>Escherichia coli</i>	
Purity	>95% as determined by SDS-PAGE. Purified by Ni-NTA chromatography.	
Unit Definition	One unit of Vaccinia Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of (α^{32} P) GTP into an 80 nt transcript in 1 hour at 37°C	
Reaction Condition	1X Capping enzyme reaction buffer, supplemented with 0.5 mM GTP and 0.1 mM S-adenosylmethionine (SAM). Incubate at 37°C. 10X Capping enzyme Reaction Buffer: 500 mM Tris-HCl (pH 8.0), 50 mM KCl, 10 mM MgCl ₂ , 10 mM DTT.	
Storage Buffer	Capping enzyme is supplied in 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100 and 50% Glycerol.	
Storage	-20°C or -80°C for 12 months under sterile conditions from date of receipt.	

Capping procedures:

1. Combine RNA and nuclease-free H₂O to a final 15 μ L.
2. Heating at 65°C for 5 minutes then chill on ice for 5 minutes.
3. Below reaction mixture should be prepared on ice and combined in the followir order:

Manuel

Component	Amount	Final concentration
Denatured RNA	15 μ L	-
10X Capping Buffer	2 μ L	1X
10 mM GTP	1 μ L	0.5 mM
SAM (2 mM)	1 μ L	0.1 mM
Vaccinia Capping Enzyme	1 μ L	10 U/rxn

4. Gently mix the reaction thoroughly to achieve uniform distribution.
5. Incubate at 37°C for 30 minutes.

Notes

Reaction should be performed under RNase free condition. Use nuclease-free tubes, reagents and water to avoid RNase contamination. Also, wear gloves when working with RNA.

For Research Use Only. Not for use in diagnostic and therapeutic procedures.