

Vaccinia Capping System



1-800-632-7799
info@neb.com
www.neb.com



M2080S 009140316031

M2080S

RR 37°

400 units **10,000 U/ml** **Lot: 0091403**
RECOMBINANT **Store at -20°C** **Exp: 3/16**

Description: Based on the Vaccinia virus Capping Enzyme, the Vaccinia Capping System provides the necessary components to add 7-methylguanylate cap structures (Cap 0) to the 5' end of RNA (1). In eukaryotes, these terminal cap structures are involved in stabilization (2), transport (3), and translation (4) of mRNAs. Enzymatic production of capped RNA is an easy way to improve the stability and translational competence of RNA used for *in vitro* translation, transfection, and microinjection. Alternatively, use

of labeled GTP in a reaction provides a convenient way to label any RNA containing a 5' terminal triphosphate.

This single enzyme is composed of two subunits (D1 and D12) and has three enzymatic activities (RNA triphosphatase and guanylyltransferase by the D1 subunit and guanine methyltransferase by the D12 subunit); all necessary for addition of a complete Cap 0 structure, m7Gppp5'N (5,6). *In vitro* transcripts can be capped in less than one hour in the presence of the capping enzyme, reaction buffer, GTP, and the methyl donor, SAM. Capping is nearly 100% efficient and all capped structures are added in the proper orientation, unlike co-transcriptional addition of some cap analogs (7).

Source: An *E. coli* strain that carries the genes for the Vaccinia (WR) capping enzyme

Applications:

- Capping mRNA prior to translation assays/*in vitro* translation
- Labeling 5' end of mRNA

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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.

Reagents Supplied with Enzyme:

| | |
|---------------------------------------|--------|
| Vaccinia Capping Enzyme (10 units/μl) | 40 μl |
| 10X Capping Buffer | 100 μl |
| GTP (10 mM) | 50 μl |
| SAM (32 mM) | 100 μl |

Reaction Conditions: 1X Capping Buffer (50 mM Tris-HCl, pH 8.0; 5 mM KCl, 1 mM MgCl₂, 1 mM DTT). Incubate at 37°C.

1X Capping Buffer:

| |
|------------------------|
| 50 mM Tris-HCl, pH 8.0 |
| 5 mM KCl |
| 1 mM MgCl ₂ |
| 1 mM DTT |

Unit Definition: One unit of Vaccinia Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of (α-³²P) GTP into an 80 nt transcript in 1 hour at 37°C.

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Quality Control Assays

RNase Assay: Incubation of a 10 μl reaction containing 10 units of Vaccinia Capping Enzyme with 40 ng of 300 mer RNA transcript for 2 hours at 37°C resulted in less than 10% degradation of RNA as determined by denaturing PAGE analysis.

Exonuclease Activity: Incubation of a 50 μl reaction containing 10 units of Vaccinia Capping Enzyme with 1 μg of a mixture of single and double-stranded [³H] *E. coli* DNA for 4 hours at 37°C released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of a 10 μl reaction containing 10 units of Vaccinia Capping Enzyme with 300 ng of supercoiled plasmid for 4 hours at 37°C produced less than 10% nicked or linear molecules as determined by agarose gel electrophoresis.

Notes on use (read prior to setting up reaction)

1. RNA used for capping reactions should be purified prior to use and suspended in nuclease-free water. EDTA should not be present and the solution should be free of salts.

(see other side)

CERTIFICATE OF ANALYSIS

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Notes on use (read prior to setting up reaction)

1. RNA used for capping reactions should be purified prior to use and suspended in nuclease-free water. EDTA should not be present and the solution should be free of salts.

(see other side)

CERTIFICATE OF ANALYSIS

- While RNase Inhibitor is not required, many users prefer to use it to enhance the stability of their RNA in solution. If this is desired, 0.5 μ l of a standard RNase inhibitor prep (such as RNase Inhibitor, Murine, NEB #M0314) can be added at the time of reaction set-up. The additional volume can be subtracted from the amount of H₂O used in step 1 of both the capping and the labeling protocols.
- Heating the solution of RNA prior to incubation with the Vaccinia Capping Enzyme removes secondary structure on the 5' end of the transcript. Extend time to 10 minutes for transcripts with known highly structured 5' ends.
- SAM is unstable at pH 7–8, 37°C and should be mixed fresh prior to starting the reaction. We recommend determining how many reactions will be performed and diluting an aliquot of the 32 mM stock to 2 mM just prior to setting up the reactions. This "working stock" should be kept on ice to prevent degradation of SAM.
- For transcripts with known structured 5' ends, the reaction time can be extended to 60 minutes to improve capping efficiency.

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- For labeling the 5' end, the total GTP concentration should be around 1–3X the molar concentration of mRNA in the reaction. The 10 mM stock can be diluted and a "spike" of hot GTP added to make the GTP mix.

Capping Protocol

This protocol is designed to cap up to 10 μ g of RNA (100 nt or larger) in a 20 μ l reaction. Reaction size can be scaled up, as needed. The system provides enough reagents to perform 40 reactions at the 10 μ g RNA/20 μ l reaction scale.

- Combine RNA and Nuclease-free H₂O in a 1.5 ml microfuge tube to a final volume of 15.0 μ l
- Heat at 65°C for 5 minutes
- Place tube on ice for 5 minutes
- Add the following components in the order specified:

| | |
|--|-----------------------------|
| Denatured RNA (from above) | 15.0 μ l |
| 10X Capping Buffer | 2.0 μ l |
| GTP (10 mM) | 1.0 μ l |
| SAM (2 mM, dilute 32 mM stock to 2 mM) | 1.0 μ l |
| Vaccinia Capping Enzyme | 1.0 μ l |
| Total Volume | 20 μl |

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| Vaccinia Capping Enzyme | 1.0 μ l |
| Total Volume | 20 μl |

- Incubate at 37°C for 30 minutes
- RNA is now capped and ready for use in downstream applications. Some applications may require RNA to be purified prior to use. If the RNA needs a poly(A) tail, NEB Poly(A) Polymerase (NEB #M0276) can be used.

Labeling Protocol

This protocol is designed to label any RNA containing a 5' triphosphate in a 20 μ l reaction. The efficiency of label incorporation will be impacted by the molar ratio of RNA: GTP, as well as the ratio of cold and hot GTP. RNA used for labeling needs to be free of cold GTP and salts. Reaction size can be scaled up, as needed. The system provides enough reagents to perform 40 reactions at the 20 μ l reaction scale.

- Combine RNA and Nuclease-free H₂O in a 1.5 ml microfuge tube to a final volume of 14.0 μ l
- Heat at 65°C for 5 minutes
- Place tube on ice for 5 minutes

- Incubate at 37°C for 30 minutes
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