Vaccinia Capping System

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

M2080S 005130515051

M2080S

RR 37°

400 units	**10,000 U/ml**	**Lot: 0051305**
RECOMBINANT	**Store at –20°C**	**Exp: 5/15**

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

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 $MgCl_2$, 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-^{32}P) GTP into an 80 nt transcript in 1 hour at 37°C.

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 [^{3}H] *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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