NEW ENGLAND BioLabs Inc.
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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *Faustovirus Capping Enzyme* |
| *Catalog Number:* | *M2081S* |
| *Concentration:* | *25,000 U/ml* |
| *Unit Definition:* | *One unit of Faustovirus Capping Enzyme is defined as the amount of enzyme required to convert 75 pmol of a 20-mer transcript to Cap-0 RNA in 30 minutes at 37°C.* |
| *Packaging Lot Number:* | *10164284* |
| *Expiration Date:* | *09/2024* |
| *Storage Temperature:* | *-20°C* |
| *Storage Conditions:* | *40 mM Tris-HCl, 100 mM NaCl, 50 mM Arginine, 0.1 mM TCEP, 50% Glycerol, (pH 8.0 @ 25°C)* |
| *Specification Version:* | *PS-M2081S/L v1.0* |

| **Faustovirus Capping Enzyme Component List** | | | |
|---|---|---|---|
| **NEB Part Number** | **Component Description** | **Lot Number** | **Individual QC Result** |
| N2080AVIAL | GTP | 10163001 | **Pass** |
| M2081SVIAL | Faustovirus Capping Enzyme | 10163002 | **Pass** |
| B9003SVIAL | S-adenosylmethionine (SAM) | 10153874 | **Pass** |
| B2181AVIAL | FCE Capping Buffer | 10163000 | **Pass** |

| **Assay Name/Specification** | **Lot # 10164284** |
|---|---|
| **Protein Purity Assay (SDS-PAGE)**<br>Faustovirus Capping Enzyme is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>A minimum of 25 units of Faustovirus Capping Enzyme is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome. | **Pass** |
| **RNase Activity (Extended Digestion)**<br>A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 25 units of Faustovirus Capping Enzyme is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection. | **Pass** |

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| Assay Name/Specification | Lot # 10164284 |
|---|---|
| **Exonuclease Activity (Radioactivity Release)**<br>A 50 µl reaction in FCE Capping Buffer containing 1 µg of a mixture of single and double-stranded [ $^3$H] E. coli DNA and a minimum of 25 units of Faustovirus Capping Enzyme incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)**<br>A minimum of 25 units of Faustovirus Capping Enzyme is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome. | **Pass** |
| **RNase Activity (Extended Digestion)**<br>A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 25 units of Faustovirus Capping Enzyme is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection. | **Pass** |
| **Exonuclease Activity (Radioactivity Release)**<br>A 50 µl reaction in FCE Capping Buffer containing 1 µg of a mixture of single and double-stranded [ $^3$H] E. coli DNA and a minimum of 25 units of Faustovirus Capping Enzyme incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **Endonuclease Activity (Nicking)**<br>A 50 µl reaction in FCE Capping Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 25 units of Faustovirus Capping Enzyme incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **Protein Purity Assay (SDS-PAGE)**<br>Faustovirus Capping Enzyme is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **Endonuclease Activity (Nicking)**<br>A 50 µl reaction in FCE Capping Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 25 units of Faustovirus Capping Enzyme incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit

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www.neb.com/trademarks for additional information.

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08 Sep 2022

Michael Tonello
Packaging Quality Control Inspector
15 Sep 2022