KRISHZYME™ Vaccinia Virus Capping Enzyme

Catalog Number: KLPL10606 / KLPL10607 / KLP10608

Description

Vaccinia virus capping enzyme is derived from a recombinant E. coli strain carries the genes for the Vaccinia capping enzyme. This single enzyme is composed of two subunits (D1 and D12) and has three enzymatic activities (RNA triphosphatase and guanylyl transferase by the D1 subunit and guanine methyltransferase by the D12 subunit).

Vaccinia virus Capping Enzyme is effective to catalyze the formation of cap structure, which can specifically attach the 7-methylguanylate cap structure (m7Gppp, Cap 0) to the 5′ end of RNA. Cap structure (Cap 0) plays an important role in mRNA stabilization, transport, and translation in eukaryotes. Capping RNA by the enzymatic reaction is an effective and simple method which can significantly improve the stability and translation of RNA for in vitro transcription, transfection, and microinjection.

Expression Host:

E.coli

Purity:

>98% as determined by SDS-PAGE quantitative densitometry by Coomassie Blue Staining.

Endotoxin:

< 20 EU/1000 units as determined by the LAL method.

Unit Definition:

One unit of Vaccinia virus Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of GTP into an 80 nt transcript in 1 hour at 37°C.

Formulation:

KRISHZYME™ Vaccinia Virus Capping Enzyme is supplied as a liquid buffer containing 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM DTT, 0.1mM EDTA, 0.1% Triton X-100, 50% glycerol.

E.coli DNA Residue:

< 0.1 pg /10 U

Bacterial Endotoxin:

<10 EU/mg, as per LAL test

Molecular Mass:

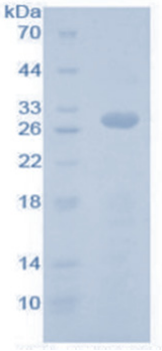
The KRISHZYME™ Recombinant Trypsin has a calculated molecular mass of ~30 kDa

Reconstitution:

Being an enzyme, the concentration may differ from lot to lot. We always recommend referring the accompanying data sheet to view the exact concentration and the recommended dilution schemata.

Centrifuge the vial at 4ºC before opening to recover the entire contents. Please contact us for any concerns or special requirements at +91-22-49198700 | Email: sales1@krishgen.com

SDS-PAGE:
Fig.1.



KDa Marker 10-80

Fig. 1. Purity analysis by SDS-PAGE Detection

Storage:

Store it under sterile conditions at -20ºC to -80ºC upon receiving for at least 12 months. It is recommended to aliquot the enzyme into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Product Instruction

Long-term storage: If the prepared recombinant cell digestion solution needs to be stored for a longer period, Store at -20°C exceeding 12 months from date of preparation.

Reaction System and Protocol

1. Capping Protocol (reaction volume: 20 ul)
   This procedure is applicable to the capping reaction of 10 ug RNA (≥100 nt) and it can be scaled up according to experimental demands.
   a. Combine 10 µg RNA and Nuclease-free H2O in a 1.5 ml microfuge tube to a final volume of 15.0 µL.
   b. Heat at 65℃ for 5 minutes followed by ice bath for 5 minutes.
   c. Add the following components in the order specified:
   d. Incubate at 37°C for 30 minutes, RNA is now capped and ready for downstream applications.

Component	Volume
Denatured RNA (≤10 µg, length≥100 nt)	15 ul
10×Capping Buffer*	2 ul
GTP (10 mM)	1 ul
SAM (2 mM)	1 ul
Vaccinia virus Capping Enzyme (10 U/ul)	1 ul
Total Reaction Volume	**20 ul**

2. 5′ terminal labelling reaction (reaction volume: 20 ul)
   This protocol is designed to label RNA containing a 5´ triphosphate and it can be scaled up according to demands. The efficiency of label incorporation will be impacted by the molar ratio of RNA: GTP, as well as the GTP content in RNA samples
   a. Combine appropriate amount of RNA and Nuclease-free H2O in a 1.5 ml microfuge tube to a final volume of 14.0 ul.
   b. Heat at 65ºC for 5 minutes followed by ice bath for 5 minutes.
   c. Add the following components in the order specified.
   d. Incubate at 37°C for 30 minutes, RNA 5′ end is now labelled and ready for downstream applications.

Component	Volume
Denatured RNA	14 ul
10×Capping Buffer	2 ul
GTP mix**	2 ul
SAM (2 mM)	1 ul
Vaccinia virus Capping Enzyme (10 U/ul)	1 ul
Total Reaction Volume	**20 ul**

*** GTP MIX refers to GTP and a small number of markers. For the concentration of GTP, refer to Usage Note 3 below.*

Application:

This product can be widely used in any experiment requiring
- Capping mRNA prior to translation assays/in vitro translation
- Labelling 5´ end of mRNA

Usage Note

1. Violent oscillation or stirring will lead to enzyme inactivation
2. The optimum temperature range of this the inhibitor was 25-55ºC, and It was inactivated at 65ºC and above.
3. The activities of RNase H, RNase 1 and RNase T1 were not inhibited by murine RNase inhibitor.
4. The inhibition of RNase activity was found in a wide range of pH (pH 5-9 were all active), and the highest activity was observed at pH 7-8.
5. Since ribonucleases typically retain activity under denaturing conditions, care must be taken to avoid denaturing RNase Inhibitor molecules which have complexed with a ribonuclease. To prevent the release of active ribonuclease, temperatures greater than 50°C and high concentrations of urea or other denaturing agents should be avoided.

References:

Vaccinia virus capping enzyme is a transcription initiation factor.
JC Vos, M Sasker, HG Stunnenberg - The EMBO Journal, 1991 - embopress.org

Versatile strategy using vaccinia virus-capping enzyme to synthesize functional 5′ cap-modified mRNAs
H Ohno, S Akamine, M Mochizuki… - Nucleic Acids …, 2023 - academic.oup.com

Crystal structure of vaccinia virus mRNA capping enzyme provides insights into the mechanism and evolution of the capping apparatus
OJP Kyrieleis, J Chang, M de la Peña, S Shuman… - Structure, 2014 - cell.com

The D1 and D12 subunits are both essential for the transcription termination factor activity of vaccinia virus capping enzyme
Y Luo, X Mao, L Deng, P Cong, S Shuman - Journal of virology, 1995 - journals.asm.org

Identification of the DNA sequences encoding the large subunit of the mRNA-capping enzyme of vaccinia virus
JR Morgan, LK Cohen, BE Roberts - Journal of virology, 1984 - journals.asm.org

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2025

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |