

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Influenza C Virus M1 [1 – 235]

Enzyme description:- ICV M1 [1 - 235]

Clone number:- DU 76132

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 70, 474.79 daltons

Average Mass 70, 519.39 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.70

Purity:- 80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.5 mM TCEP

Storage temperature:- -70 °C

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Clone Data Sheet

Influenza C Virus M1 [1 – 235]

<u>Protein</u>	ICV M1 [1 - 235]
<u>Clone number</u>	DU 76132
<u>Species</u>	Influenza C virus (ICV) strain C/Johannesburg/1/66
<u>Tags</u>	N-terminal MBP
<u>Bacterially expressed protein</u>	MMKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF PQVAATGDGPDIIFWAHDREFGGYAQSGLLAEITPDKAFAQDKLYPFTWDA VRYNGKLIAYPIAVEALSLIYNKDLPNPPKTWEETPALDKELKAKGKS ALMFNLQE PYFTWPLIAADGGYAFKYENGKYDIKVGVNDAGAKAGLT LVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYG VTVLPTFKGQPSKPFVGVLISAGINAASPNKELAKEFLENYLLTDEGLEA VNKDKPLGAVALSKYEEELVKDPRIAATMENAQKGEIMPNIPQMSAFWY AVRTAVINAASGRQTVD EALKDAQTNSSNNNNNNNNNLGDDDKVPE <u>FLEVLFQGPLGSMAHEILIAEAE AFLKNVAPETRTAIISAITGGKSACK</u> <u>SAAKLIKNEHLPLMSGEATTMHIVMRCLYPEIKPWKKASDMLNKATSSL</u> <u>KKSEGRDIRKQMKAAGDFILGVESMMKMRAFRDDQIMEMVEEVYDH PDDY</u> <u>TPDIRIGITITAWLRCKNKSERYRSNVSESGRTALKIHEVRKASTAMNE</u> <u>IAGITGLGEEALS LQRQTESLAILCNHTFGSNIMRPHLEKAIKGVEGRV</u> <u>GE</u>
<u>Native sequence</u>	Amino acids M1 – E235 (end residue is K242) of ICV M1 protein. Residue M404 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 – 367.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 393 - 400