

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Influenza C Virus NS1 [63 – 246]

Enzyme description:- ICV NS1 [63 - 246]

Clone number:- DU 76142

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 64, 806.63 daltons

Average Mass 64, 847.83 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.40

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 NS1 [63 – 246]

<u>Protein</u>	ICV NS1 [63 - 246]
<u>Clone number</u>	DU 76142
<u>Species</u>	Influenza C virus (ICV) strain C/Johannesburg/1/66
<u>Tags</u>	N-terminal MBP
<u>Bacterially expressed protein</u>	MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFP QVAATGDGPDIIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAV RYNGKLIAYPIAVEALSIIYNKDLLPNPPKTWEEIIPALDKELKAKGKSA LMFNLQEPYFTWPLIAADGGYAFKYENGGYDIKDVGVNAGAKAGLTFV VDLIKKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGV TVLPFTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELVKDPRIAAATMENAQKGEIMPNIPOMSAFWYA VRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNLGDGDDDKVPEF <u>LEVLFGQPLGS</u> GELLFNGTILQTESPTMTPASVEMKGGKFPIDFAPSNI APIGQNPIYLSPCI PNFDGNVWEATMYHHRGATLTKTMNCNCFQRTIWC HPNPSRMRLSYAFVLYCRNTKKICGYLIAKQVAGIETGIRKCFRCIKSG FVMATDEISLTILOSIKSGAQLDPYWGNEKPDIDKTEAYMLSLREAGP
<u>Native sequence</u>	Amino acids G63 – P246 (end residue) of ICV NS1 protein. Residue G404 of the fusion protein is equivalent to G63 of the native enzyme. The MBP tag is located at residues 1 – 367.
<u>Protease cleavage</u>	PreScission (<u>LEVLFGQP</u>) residues 393 - 400