

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Influenza A Virus NS1 [1 – 231]

Enzyme description:- IAV NS1 [1 - 231]

Clone number:- DU 70823

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 70, 061.81 daltons

Average Mass 70, 105.71 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.20

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 A Virus NS1 [1 – 231]

<u>Protein</u>	IAV NS1 [1 - 231]
<u>Clone number</u>	DU 70823
<u>Species</u>	Influenza A virus (IAV) strain A/Puerto Rico/8/1934(H1N1) ('PR8')
<u>Tags</u>	N-terminal MBP
<u>Bacterially expressed protein</u>	MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFP QVAATGDGPDIIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAV RYNGKLIAYPIAVEALSIIYNKDLLPNPPKTWEEIIPALDKELKAKGKSA LMFNLQEPYFTWPLIAADGGYAFKYENGGYDIKDVGVNAGAKAGLTF VDLIKKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWNSIDTSKVNYGV TVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYYEELVKDPRIAAATMENAQKGEIMPNIPOMSAFWYA VRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNNLGDDDDKVPEF <u>LEVLFQGP</u> LGSMDPNTVSSFQVDCFLWHVVRKRVADQELGDAPFLDRLRR DQKSLRGRGSTLGLDIKTATRAGKQIVERILKEESDEALKMTMASVPAS RYLTDMTLEMSRDWSMLIPKQKVAGPLCIRMDQAIMDKNIILKANFSV IFDRLETLILLRAFTEEGAIVGEISPLPSLPGHTAEDVKNVAVGLIGGL EWNNTVVRVSETLQRFWRSSNENGRPPLTPKQKREMAGTIRSEV
<u>Native sequence</u>	Amino acids M1 – V231 (end residue) of IAV NS1 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