

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of Influenza A Virus NEP [11 – 121]

Enzyme description:- IAV NEP [11 - 121]

Clone number:- DU 73915

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose Resin

Calculated molecular mass:-

Monoisotopic 57, 548.20 daltons

Average Mass 57, 548.27 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.94

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 NEP [11 – 121]

<u>Protein</u>	IAV NEP [11 - 121]
<u>Clone number</u>	DU 73915
<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 RYNGKLIAYPIAVEALSIIYNKDLLPNPPKTWEEIPALDKELKAKGKSA LMFNLQEPYFTWPLIAADGGYAFKYENGGYDIKDVGVNAGAKAGLTF VDLIKKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWNSNIDTSKVNYGV TVLPFTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAV NKDKPLGAVALKSYEEELVKDPRIAATMENAQKGEIMPNI PQMSAFWYA VRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNNLGDDDDKVPEF LEVLFQGPLGSDILLRMSKMQLESSSEDLNGMITQFESLKLYRDSLGEA VMRMGDLHSLQNRNEKWREQLGQKFEEIRWLIIEVVRHKLKITENSFEQI TFMQALHLLL EVEQEIRTFQSLI
<u>Native sequence</u>	Amino acids D11 – I121 (end residue) of IAV NEP protein. Residue D404 of the fusion protein is equivalent to D11 of the native enzyme. The MBP tag is located at residues 1 – 367.
<u>Protease cleavage</u>	PreScission (LEVLFQGP) residues 393 - 400