

## *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 73894

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 40, 143.47 daltons

Average Mass 40, 169.52 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.51

**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)**

<b><u>Protein</u></b>	IAV NEP (11 – 121)
<b><u>Clone number</u></b>	DU 73894
<b><u>Species</u></b>	Influenza A virus (IAV) strain A/Puerto Rico/8/1934(H1N1) ('PR8')
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL <u>FQGPLGS</u> <b>DILLRMSKMQL</b> <b>ESS</b> <b>SEDLN</b> GMITQ <b>FES</b> SLKLYRDSLGEAVMRMGDLHSLQNRNEKWREQLGQ <b>KF</b> <b>EEIRW</b> LIEEVRHKLKITENSFEQITFMQALHLLLEVEQEIRTF <b>SFQLI</b>
<b><u>Native sequence</u></b>	Amino acids D11 – I121 (end residue) of IAV NEP protein. Residue D232 of the fusion protein is equivalent to D11 of the native enzyme. The GST tag is located at residues 1 – 220.
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVL</u> FQGP) residues 221 - 229