Centre for AIDS Reagents

Data Sheet

DESCRIPTION: Recombinant Nef protein (C-clade consensus sequence)

REPOSITORY REFERENCE: ARP693.1
ARP693.2 (Low Endotoxin)

CHARACTERISTICS: Recombinant Nef-C protein is expressed as His tag-fusion protein in E. coli using expression vector pET24 and purified using Ni-agarose columns (Qiagen). Endotoxins were removed by phase separation using Triton X-114 (described by Liu et al. *Clinical Biochem.* 30, 1997, p. 455)

SOURCE: FIT Biotech Oyj Plc Eesti Filiaal, Tartu, Estonia

PRESENTATION: 50ug. In PBS +10% glycerol
(ARP693.2 may contain traces of Triton X-114).

NOTE: Prepared for AVIP collaborative study. Non AVIP members are restricted to 1 vial per request.

PURITY: See Fig.1 for SDS-PAGE and Fig.2 for Western blot analysis.

STORAGE: Store at –70 °C or less. Avoid multiple freeze-thaw cycles as product degradation may occur.

ACKNOWLEDGEMENTS: Publications should acknowledge the donor of the reagent and the Centre for AIDS Reagents. Suggested wording can be found on our website in the "Acknowledgement" section at:-

www.nibsc.ac.uk/spotlight/centre_for_aids_reagents.aspx

Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR, this can be by e-mail or printed copy.

PROTEIN SEQUENCE:

MGGKWSKCSIVGWPAVRERMRRTEPAAEGVGAASQDLDKHGALTSSNTAANNADCAWLEAQEDEEE
VGFPVRPQVPLRPMTYKAAFDLSFFLKEKGGLEGLIYSKKRQEILDLWVYHTQGYFPDWQNYTPGPGV
RYPLTFGWCFKLVPVDPREVEEANEGENNCLLHPMSQHGMEDEHREVLKWKFDSHLARRHMARELH
PEYYKDCLEHHHHHH

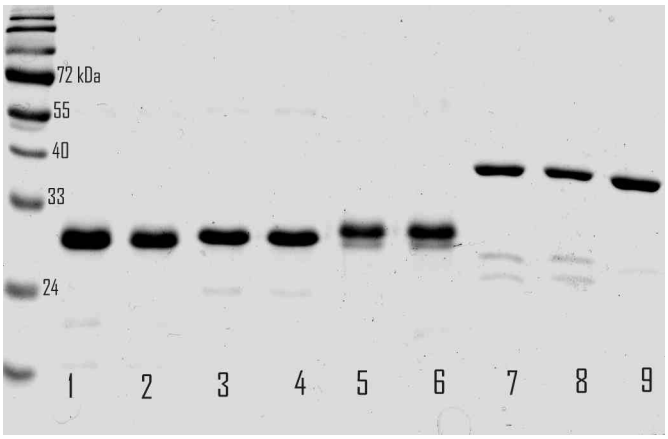

Figure 1. SDS-PAGE analysis of purified proteins (Coomassie staining)

Lane 1- Nef-A,	1 µg
Lane 2- Nef-A low endo,	1 µg
Lane 3- Nef-B	1 µg
Lane 4- Nef-B low endo	1 µg
Lane 5- Nef-C	**1 µg**
Lane 6- Nef-C low endo	**1 µg**
Lane 7- p17/24-B	1 µg
Lane 8- p17/24-B low endo	0.8 µg
Lane 9- p17/24-C	1 µg

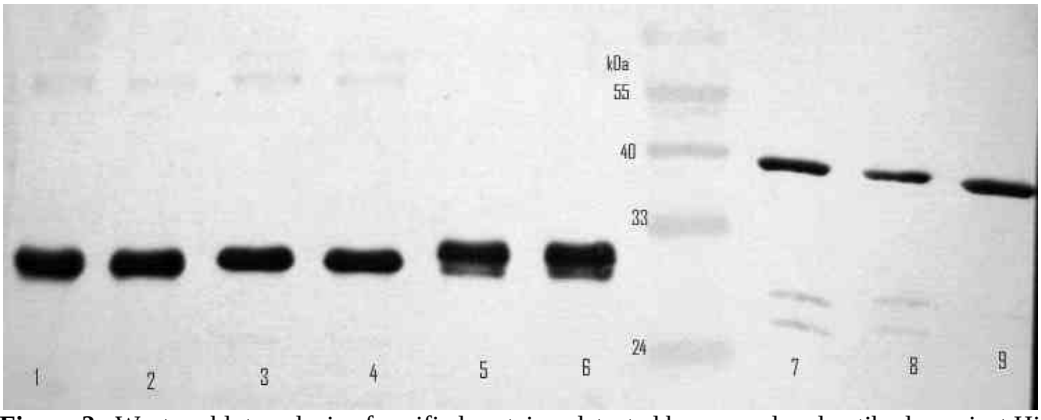

Figure 2. Western blot analysis of purified proteins, detected by monoclonal antibody against His-tag.

Lane 1- Nef-A	0.5 µg
Lane 2- Nef-A low endo	0.5 µg
Lane 3- Nef-B	0.5 µg
Lane 4- Nef-B low endo	0.5 µg
Lane 5- Nef-C	**0.5 µg**
Lane 6- Nef-C low endo	**0.5 µg**
Lane 7- p17/24-B	0.5 µg
Lane 8- p17/24-B low endo	0.4 µg
Lane 9- p17/24-C 1 µg	0.5 µg