Centre for AIDS Reagents.

Data Sheet

NAME :	Baculovirus expressing HIV-1 HXB2 gp120 with FLAG tail
REPOSITORY REFERENCE :	**ARP2114 - 2115**
PROVIDED:	0.5ml
REFERENCE :	Lia et al (2000, J Exp Med **192**: 587-593
ACKNOWLEDGEMENTS :	Publications should acknowledge the donor of the reagent and the Programme EVA Centre for AIDS Reagents. Suggested wording can be found on our website at http://www.nibsc.ac.uk/spotlight/aidsreagent/index.html in the "Acknowledgements" section. Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR (this can be electronically or as a paper copy)

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METHODS:

1. Purification of virus stock:

Sf9 cells can be used with serum free medium SF900-II SFM (GIBCO) to grow cells to a density of 5 x 10^5 cells/ml, the virus prep of m.o.i 0.1 will be used. The infected suspension culture is kept for 6-7 days at 28ºC. The supernatant should be clarified by centrifugation at 3,000 rpm and used as a virus stock. The virus titres should reach $10^{7.5-8}$ PFU.

2. Protein Production:

Use serum free SF9 cells and infect the suspension culture of density 1-2 x 106 with m.o.i of 5. The infected suspension is being kept at 28C for 3 days. The infected supernatants are clarified at 3200 rpm for 30 mins. The supernatant itself is ready to load on an M2 Mab column (M2 from sigma Cat No. A1205). The column equilibriated with TBS buffer (TBS Buffer: 50mM Tris-HCL, 150mM NaCl, pH7.4).

3. Purification:

Pass the supernatant through M2 column twice (or more) and wash the column with TBS buffer 3 times of column volume. Elute the protein with 0.1M glycine-HCl pH3.5. The eluted fractions are neutralised with 1M pH8.0 Tris immediately as used in any other antibody column., Pool the fractions and the gp120 eluted is very pure, over 90% judged by SDS PAGE and gel filtration (Hardly any contaminated proteins). Therefore it can be directly used.

CONSTRUCTS: Vector p2Bac baculovirus expression system (invitrogen) cloned by BamH1/HindIII

FLAG TAIL: **gp120 - EFGGDYKDDDDKGG**