Centre for AIDS Reagents.

Data Sheet.

NAME :	HIV-nef /MHC Tetramers
REPOSITORY REFERENCE :	ARP7077.1-15
DESCRIPTION OF REAGENT :	See below
CHARACTERISTICS :	See below
PRESENTATION and STORAGE:	MHC/peptides should be stored frozen under liquid nitrogen vapour. The streptavidin should be stored unfrozen at 4C.
SOURCE :	Dr Dirk Busch
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)

IMPORTANT: **Include datasheets for individual lots from file stored in 3097!**

National Institute for Biological Standards and Control – Assuring the quality of biological medicines
Blanche Lane South Mimms Potters Bar Hertfordshire EN6 3QG United Kingdom
Tel +44 (0)1707 641000 Fax +44 (0)1707 641050 www.nibsc.ac.uk
A World Health Organization Laboratory for Biological Standards

Generation of HIV-nef/MHC Tetramers / Programme EVA

Dirk H. Busch
Institute for Microbiology, Immunology and Hygiene

Prof. Volker Erfle
Institute of Virology

Technical University Munich
Trogerstr. 4a/b
81675 Munich Germany

Immune responses against regulatory proteins of HIV, like HIV-nef, may be important in the control of infection, disease progression and protective immunity. Therefore, these proteins became interesting candidates as targeting antigens for the development of HIV-vaccines. However, a detailed quantitative and qualitative analysis of specific T cell responses to these antigens is necessary to determine their involvement in disease control, as well as for the interpretation of vaccination studies. This would be best achieved by the MHC tetramer technology, since this method detects specific T cells independent of their functional status with a high sensitivity and specificity. We therefore generated HIV-nef/MHC tetramer reagents together with "Programme EVA".

Soluble and biotinylated MHC molecules were successfully generated for all 15 different HIV-nef epitopes (see table). Although yields varied substantially between different reagents, we were usually able to reach the intended total amount of soluble MHC reagents (≅ > 2mg SA-PE tetramer reagent).

Table 1

epitope	AS-sequence	Restriction element	Reference
Nef (13-20 LAI)	WPTVRERM	HLA-B8	Goulder et al (1997)
Nef (68-77 LAI)	FPVTPQVPLR	HLA-B7	Haas et al. (1996)
Nef (72-80 SF2)	FPVRPQVPL	HLA-B35	Tomiyama et al. (1997)
Nef (75-85 SF2)	RPQVPLRPMTY	HLA-B35	Tomiyama et al. (1997)
Nef (73-82 LAI)	QVPLRPMTYK	HLA-A11	Couillin et al. (1994)
Nef (73-82 LAI)	VPLRPMTY	HLA-B35	McMichael (1994)
Nef (77-85 LAI)	RPMTYKAAL	HLA-B7	Bauer et al. (1997)
Nef (84-92 LAI)	AVDLSHFLK	HLA-A11	McMichael (1994)
Nef (89-97 LAI)	FLKEKGGL	HLA-B8	Price et al. (1997)
Nef (105-114 LAI)	RRQDILDLWI	HLA-B27	Goulder et al. (1997)
Nef (128-137 LAI)	TPGPGVRYPL	HLA-B7	Haas et al. (1996)
Nef (133-141)	YPLTFGWCY	HLA-B35	Shiga et al. (1996)
Nef (136-145)	PLTFGWCFKL	HLA-A2	Durali et al. (1998)
Nef (180 189 LAI)	VLEWRFDSRL	HLA-A2	Liebermann et al. (1997)
Nef (186-193 LAI)	DSRLAFHH	HLA-B35	Hadida et al. (1995)

We originally planed to multimerize MHC molecules directly with strepatavidin-PE (SA-PE) and to store aliquots longterm in liquid nitrogen. Unfortunately, more recent studies regarding the stability of MHC tetramers containing SA-PE as backbone show that they are not very stable in N_2 (mostly a stability problem of the PE fluorescence). However, MHC/peptide complexes itself can be frozen in N_2 and appear to remain stable for a long time. Therefore, we adopted the following strategy for the distribution of HIV-nef/MHC tetramer reagents through NIBSC: Aliquots of biotinylated MHC/peptide complexes (150µg, frozen at < -80°C) were sent to NIBSC. The potential user can later by himself thaw the samples and multimerize MHC molecules by addition of SA-PE. Since the quality of SA-PE is a very crucial aspect of MHC tetramer reagents (even batches from the same company can differ quite dramatically) and since we promised to provide "complete tetramer reagents" for "Programme EVA", we sent "tested" SA-PE together with the monomers to NIBSC. Each aliquot will result in approx. 400µg MHC tetramer reagent (for staining approx. 1-5µg per 1 x 10^6 cells will be required).

The SA-PE aliquots (250ug) necessary for multimerization of the biotinylated MHC
Molecules are stored separately at +4C. Each tube is labeled with the corresponding Lot-number of the frozen MHC monomer sample. The amber tubes are pre-labelled (ideal for storage of MHC/SA-PE reagents) already with the exact tetramer they contain. To provide MHC-tetramer reagents for other labs, Programme EVA will send out the frozen vial with the biotinylated MHC/peptide complexes (on dry ice!!) and the SA-PE vial with the matching Lot# (at 4°C, do not freeze the SA-PE!!!). Together with the protocol (copies for each Lot# are already prepared) for the last multimerization step to generate SA-PE tetramers, it should be very simple for the user to get started working with the MHC tetramers.