Centre for AIDS Reagents.

Data Sheet.

DESCRIPTION:	Biotinylated anti-p24
REPOSITORY REFERENCE:	ARP454
ORIGINATORS DESIGNATION:	EH12-BT #267220
SPECIFICITY:	HIV-1 p24
IMMUNOGEN:	Inactivated HIV-1_{CBL-1}
DESCRIPTION:	Biotinylated Mab to p24 (see ARP313).
CHARACTERISTICS:	Suitable for use in ELISA'S for p24 as a detection antibody.
PRESENTATION:	100ul aliquots (2.32mg/ml), 7.87 moles biotin/mole IgG. Contains PBS + Sodium Azide.
STORAGE:	2-8^{o}C
SOURCE:	Dako Ltd
ACKNOWLEDGEMENT:	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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D.I.Y HIV p24 ELISA

(ARP454)

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MATERIALS:

Empigen solution. This is a 30% solution of a zwitterionic detergent available from Calbiochem (#324690) in 100g lots. Store @4°C.

Tween 20 solution. This is a 10% solution of a non-ionic detergent available from Calbiochem (#655206) in 50ml lots. Store at -20°C.

Microelisa plates. Costar 8-well strips (#C2580, 800/case, £128, from Northumbria Biologicals: ~£2/plate).

Capture antibody. Affinity-purified sheep anti-HIV-p24 gag. Code # D7320 from Aalto Bioreagents, 14 Main Street, Rathfarnham, Dublin 14, Eire, tel Eire (01) 900685/901264, fax (01)-900122 or obtainable from CFAR , code ARP410. It comes lyophilized and should be reconstituted in 2ml water, aliquoted at 100µl and stored @ -20°C.

Coating buffer. 100mM NaHCO3, pH 8.5

10xTBS. 1.44M NaCL, 0.5% Tween 20 (50ml Tween 20 solution per 1/buffer) 250mM Tris pH7.5. Aliquot at 100ml and autoclave. Add 1ml of 25% NaN3 per 100ml 10xTBS and then dilute 1:10 on day of use.

Milk solution. Dissolve 0.4g of Cadbury's Marvel skimmed milk powder in 20ml 1xTBS. Make up fresh each time.

PBS/E/S. PBS containing 0.1% Empigen and 10% serum.

p24 standards. Baculovirus-derived p24 is obtained from Ian Jones at 125µg/ml.It is diluted to 1µg/ml in PBS/E/S and stored in 200µl aliquots at -20°. Half-log serial dilutions are made using 100µl PBS/E/S diluent and 46.25µl transfer volume (seven samples to 1ng/ml).

TMT/SS. 1xTBS containing 2% Marvel, 20% sheep serum (SeraLab), 0.5% Tween 20: (6.5ml water + 1ml 10xTBS + 0.2g Marvel + 2ml sheep serum + 0.5ml Tween 20 solution).

Detection antibody. Biotinylated monoclonal mouse anti-HIV-p24 gag (ARP454). The ascities fluid from hybridoma EH12-E1 (Fearns & Tedder) was obtained from the MRC ARP, purified by three rounds of NaSO4 selective precipitation and then by ion-exchange chromatography and biotinylated using biotin[linker]-N-hydroxysuccinimide ester. This is provided as a 0.1mg/ml solution (1000x) in TMT/SS and is stored at -70°C. A suitable method of storage is 0.1ml aliquots of a 1:10 TMT/SS dilution (sufficient for 1 plate) at -20°. For use, dilute a further 1:100 with TMT/SS.

Streptavidin-alkaline phosphatase. From Boehringer Mannheim (cat #1089 161). £99 for 1000u (1ml). Store at 4°. Dilute 1:1000 with TMT/SS.

PNPP. Add one 5mg tablets of p-nitrophenyl phosphate (Sigma #N9389) to 5ml reaction buffer (10mM ethanolamine, 0.5mM $MgCl^2$, pH9.8 with HCl) just before use. (sufficient for 6 columns – 48 wells).

METHOD

1.*Preparing the plate*: For 96 wells, add 100µl capture antibody to 9.9ml coating buffer. Add 100µl to each well of the microelisa plate, seal the wells with semi-transparent tape and incubate at r/t o/n.

2.*Wash 1*. All washes are done using the Titertek plate washer set at 6x200µl washes per well and 1xTBS as the washing buffer.

3.*Block*. Add 200µl milk solution to each well and incubate r/t for 30mins (seal wells).

4.*Wash 2*. The plates or strips of wells can be sealed with tape and stored for some time at 20ºC until required. Allow to warm up thoroughly before proceeding.

5. *Viral lysate*. Transfer approx 0.5ml of t/c supernatant from infected cells to a microfuge tube and clarify by centrifugation at low speed (6500rpm) for 10min.Transfer 270µl of supernatant to a fresh tube containing 30µl 1% Empigen solution. Mix and heat at 56ºC for 30mins. Store sample at -20ºC, it is now safe for use outside the containment lab but wipe outside of the tube first with 70% ethanol.

6.*Antigen capture*. Add 100µ1 of virul lysate to each well. The neat lysate is usually adequate for the method described here since a strong virus culture will be detected by the first substrate and very weak cultures will be picked up by the amplification step. If one wishes to use the amplification step directly, dilutions may be made in PBS/E/S. Seal and incubate overnight at room temp.

7.*Wash 3.*

8.Antigen detection. Add 100µl diluted detection antibody to each well and incubate for 2hrs at r/t.

9.*Wash 4.*

10.Streptavidin. Add 100µl of diluted streptavidin-alkaline phosphatase to each well and incubate 1hr at r/t.

11.*Wash 5.*

12.Chromogenesis. Add 100µl pNPP solution to each well and incubate at r/t. Read absorbance at 405nm on Titertek plate reader ar, say, 1min, 5min, 20min, 1hr.

1.This method is adapted from that of J.Moore, Chester Beatty Labs. It involves the use of a sheep polyclonal antibody raised against 3 HIV-1 gag peptides to capture antigen and a biotinylated monoclonal antibody raised against 2 different gag peptides followed by streptavidin-alkaline phosphatase to detect bound antigen. In the original assay, the second antibody was directly conjucgated to alkaline phosphatase and a subsequent amplification step was used to increase the sensitivity of the AP reaction. Amplification is supposed to provide linear results over the range 0.3- 10ng/ml in the sample. In my hands, this method gave a very steep response over the range 1-25ng/ml which was subject to a considerable amount of error variation. I found that by omitting the amplification step and extending the capture step to O/N, a reliable response in the range 10-100ng/ml could be obtained. This was adequate for most experiments involving lymphocyte-tropic strains but was limiting for work with monocytes. Accordingly, I have obtained a large quantity of the second antibody in its unconjugated form from the ARP and have purified and biotinylated the Ig fraction. This reagent gives a good response over the range 1-1000ng/ml and is available in large quantities. I recommend that the plate is read once after 5min and again after about 60min to ensure that both high and low values are measured.