



*HIV-1-p24 ANTIGEN ELISA PROTOCOL

Introduction :

The assay is a twin-site sandwich ELISA (Ref :Moore et al., 1990, 1991). Briefly, p24 antigen is captured from a detergent lysate of virions onto a polyclonal antibody adsorbed onto a solid phase. Bound p24 is detected with an alkaline phosphatase-conjugated anti-p24 monoclonal antibody and the AMPAK ELISA amplification system. This assay is in routine use at the Aaron Diamond Center and elsewhere in the USA and Europe for monitoring the rate of HIV production in tissue culture. Further details are presented in Patience et al., (1991).

Antibodies :

Product **code D7320**, supplied by Aalto Bio Reagents, Dublin, Ireland, [Tel: +353-1-4900685 ; FAX : +353-1-4900122] is a mixture of three sheep polyclonal antibodies raised against peptides from the HIV-1 (LAV-1) sequence, then affinity-purified against the immunogenic peptides. The amino acid sequences used are :

SALSEGATPQDLNTML	aa 173-188
GQMREPRGSDIA	aa 226-237
LDIRQGPKPEFRDYV	aa 283-297

These sequences are substantially conserved between HIV-1 isolates.

Product **code BC 1071** (clone EH12E1), is a monoclonal antibody raised against the HIV-1 (CBL-1) isolate (Ref: Weiss et al., 1985,1991) by Bridget Ferns, Richard Tedder and colleagues (Ref: Spence et al., 1989) and mapped to a complex epitope incorporating two distinct peptide sequences (Ref: Ferns et al., 1990).as follows :
GHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQ (aa 193-227) and
NPPIPVGEIYKRWII (aa 253-267).These regions of p24 are conserved between HIV-1 strains, and also substantially between HIV-1 and HIV-2.

The alkaline phosphatase conjugate of **BC 1071** (clone EH12E1), **code BC 1071-AP** is available from Aalto Bio Reagents.

Detection system for Alkaline Phosphatase label antibody :

AMPAK Detection System, product code **K6200**, manufactured by Oxoid (Ely) Ltd. and distributed by Aalto Bio Reagents.

*Devised by Dr. John Moore, now working at Cornell University, New York

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltoioreagents.ie Web:www.aaltoioreagents.ie



I.S. EN ISO 9001:2000





Methods

1. D7320 for HIV-1 is reconstituted in water at 1 mg / ml and stored frozen in 100µl aliquots. It is coated onto Immulon II microelisa plates (Dynatech Ltd.) by incubating them overnight at room temperature. Seal the plates with cling film. The optimal antibody concentration is 5-10µg / ml, and the coating buffer is 100µl of 100mM NaHCO₃, pH 8.5. Check that the pH of the bicarbonate buffer is below 9.0, as the stock solution tends to drift alkaline over a few weeks because of carbon dioxide absorption. Above about pH 9.5, the stability of the antibody is compromised. Because of variation in coating efficiency, it may be advisable to use only the central 60 or fewer wells for assay and fill the peripheral wells with blank buffer during coating.
2. The plates are washed twice with 200µl of TBS (144mM NaCl, 25mM Tris, pH 7.5).
3. HIV is routinely inactivated by adding Empigen zwitterionic detergent (supplied by Sigma ;Empigen BB, Catalog No. 45165) to a final concentration of 1% by volume, followed optionally by incubation at 56°C for 30 minutes. The lysates can then be stored frozen if necessary. The amount of lysate to add to wells varies between HIV strains and is a matter of experience. A reasonable estimate is to add 0.01-0.1µl (ie. 100µl of 1:1000 to 1:10,000 dilution) of high-titre HIV-1 stock such as LAV-1. The antigen capture works best at a final Empigen concentration of 0.05-0.1%, so serial dilutions are made in TBS / 0.05% Empigen and the volume of the wells adjusted to 100µl with the same buffer. The capture stage is complete within 2-3 hours at room temperature. Capture of p24 by D7320 is seriously inhibited by detergents such as Tween or NP40 or by the omission of detergent. The best detergent for this assay is Empigen at 0.05-0.1%. Since virus samples are usually inactivated in 1% Empigen, the p24 standard is diluted initially into 1% Empigen (see below) before construction of a calibration curve in 0.1% Empigen.
4. Unbound p24 is washed away with 2 x 200µl of TBS and bound p24 detected with pre-diluted BC 1071-AP (100 µl / well). This pre-dilution is at 1:1000 to 1:3000 (approximately 0.5µg / ml) in TMT / SS [2% Marvel fat free skimmed milk powder, 20% sheep serum, 0.5% Tween 20 in TBS] and binding is complete within 30-60 minutes at room temperature.

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltoioreagents.ie Web: www.aaltoioreagents.ie





5. Unbound BC 1071-AP is removed by 4 washes with 200µl AMPAK wash buffer, diluted 1:20 in water as specified by the manufacturers, Oxoid (Ely) Ltd.
6. AMPAK is used as specified in the kit insert except that 50µl of reconstituted substrate solution is added for 1 hour, followed by 50µl of reconstituted amplifier for a timed 5-10 minutes. The reaction is stopped with 50µl of acid and the plates read at 492nm.
7. The HIV-1 p24 assay is calibrated using known amounts of purified recombinant p24 protein, eg **code AG 6054** available from Aalto Bio Reagents (see ref : Mills and Jones, 1990). For optimal precision this should be detergent treated (and heated if appropriate) in the same way as the virus samples. A p24 standard is best stored in small (10-20µl) aliquots in TBS / 10% serum at -20°C. Each aliquot is used once only. Alternatively, calibration can be achieved by using frozen aliquots of a high-titre virus lysate as a reference standard for each assay. This will enable relative values to be determined within an assay.
8. Under optimal conditions it is possible to detect down to 3pg of recombinant HIV-1-p24 in a 100µl microtitre well, although the assay is most sensitive between 30-1000pg / well. At approximately 1 fg core protein per retrovirus (ref : Vogt, 1965), the detection limit corresponds to 1000-3000 virions. The HIV-1 assay is therefore of comparable sensitivity to second generation commercial kits (eg Abbott, Coulter, DuPont). The assay has been optimised for p24 antigen detection in human serum, and there may be a reduction in performance. If the coated plates are prepared the night before, results are easily obtainable within a working day. Exclusive of time to dispense solutions in wells, the capture stage takes 2-3 hours (the longer, the more sensitive), the conjugate stage about 1 hour, and the AMPAK stage about 1 hour. Although other versions of this assay may be slightly cheaper, they are much slower and less sensitive.

A typical calibration curve is attached. The calibrant used was baculovirus p24 (ref : Mills and Jones, 1990). For most purposes, variation in absolute p24 is not critical: as interest is focussed more on relative values.

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltobioreagents.ie Web: www.aaltobioreagents.ie



I.S. EN ISO 9001:2000





References

Ferns R.B., Partridge J.C., Spence R.P., Hunt N. and Tedder R. S. (1989). AIDS 3 : 829-834

Mills H.R. and Jones I.M. (1990). AIDS 4 : 1125-1131

Moore et al (1990-1991). The assay is described in SCIENCE 250 : 1139-1142 (1990) and J.VIROL 65 : 852-860 (1991).

Patience C., Moore J.P. and Boyd M. (1991) Methods in Molecular Biology Vol 8 : Practical Molecular Virology: The Humana Press Inc., Clifton NJ pp 131-140.

Spence R.P., Jarvill W.M., Ferns R.B., Tedder R.S. and Parker D. (1989). J.GEN VIROL 70 : 2843-2851.

Vogt P.K. (1965). Avian Tumor Viruses, in ADVANCES IN VIRUS RESEARCH , Vol 2 (K.M.Smith and M.A. Lauffer, eds) pp294-385.

Weiss R.A., Clapham P.R., Cheingsong-Popov R., Dalglish A.G., Carne C.A., Weller I.V.D. and Tedder R.S. (1985). NATURE 316 : 69-72 and NATURE 349 : 374(1991).

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

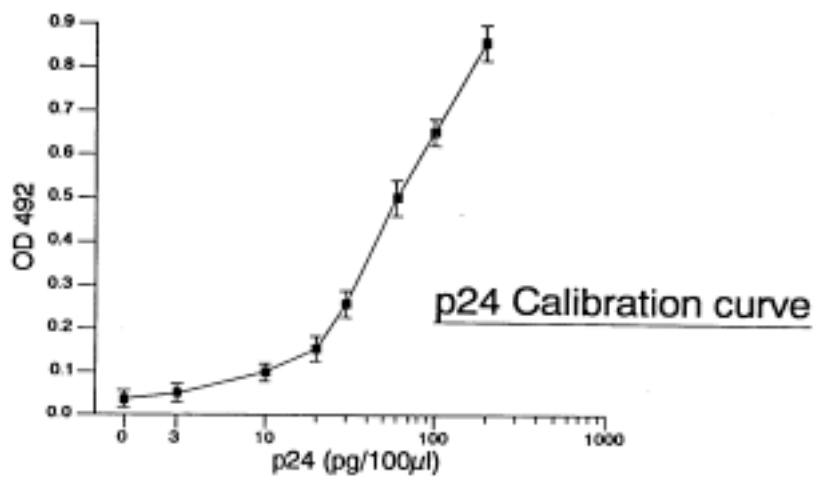
E mail: info@aaltoioreagents.ie Web: www.aaltoioreagents.ie



I.S. EN ISO 9001:2000



AALTO



Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltobioreagents.ie Web: www.aaltobioreagents.ie



I.S. EN ISO 9001:2000