



HIV-1 gp120 Standard :

Recombinant HIV-1 gp120 Antigen, product **code BR 6106**, supplied by Aalto Bio Reagents, Dublin, Ireland, [Tel: +353-1-4900685; FAX : +353-1-4900122].

Methods :

1. D7324 is reconstituted in distilled water at 1mg/ml and stored in frozen aliquots.
2. D7324 is coated onto Immulon II microelisa plates (Dynatech Ltd.) by incubating them for 12-18 hours at room temperature in 100µl per well of 100mM NaHCO₃, 1mM EGTA, pH 9.6. The optimal antibody concentration is 5µg /ml.
3. The wells are washed twice with 200µl of Tris-Buffered Saline (TBS; 144mM NaCl, 25mM Tris, pH 7.6), blocked for 30 minutes with 200µl of a solution of 2% non-fat milk powder (Marvel, Cadbury Ltd.) in TBS, and then washed again with TBS.
4. Sample is added to the wells in 100µl of TBS (a detergent such as 1% NP40 can be added but it is usually not necessary) and incubated for 2 hours at room temperature.
5. Unbound protein is removed washing twice with TBS (200µl) and captured gp120 is detected by addition for 1 hour of a second antibody, as outlined above. The antibody is diluted in TMT/SS buffer (4% nonfat milk powder and 0.5% Tween-20 in TBS plus 20% sheep serum, 100µl per well). The concentration of detection antibody to be used must be determined empirically, e.g., by titration.
6. Unbound antibody is removed by washing the wells three times with TBS (200µl), then the AP- labelled detection antibody is added for 1 hour in TMT/SS buffer.

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7. Unbound antibody –AP is removed by washing the wells six times with 200µl of AMPAK wash buffer, and bound antibody –AP is detected with the AMPAK-II ELISA amplification system (Dako Diagnostics) essentially as recommended by the manufacturer^{2,3}. The reactions are stopped with 50µl of 0.5 M HCl and the absorbance determined at 492nm. Alternative detection systems may also be suitable, as noted above.
8. The HIV-1 gp120 assay is calibrated using known amounts of purified recombinant HIV-1 gp120 Antigen (product code BR 6106). It is recommended to assign a formal HIV-1 gp120 concentration for product code BR 6106 with reference to a commercial assay for which formally assigned standards are available.

References :

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4. Ratner L Haseltine, W Patarca R et al: Complete Nucleotide Sequence of the AIDS Virus, HTLV-III Nature, 313, 277-284 (1985).
5. J.P.Moore and J.Sodroski: Antibody cross-competition analysis of the human immunodeficiency virus type 1 exterior envelope glycoprotein. J.Virol. 70, 1863-1872 (1996).
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7. J.P.Moore, Y.Cao, J.Leu, L.Qin, B.Korber and D.D.Ho. Inter- and intraclade neutralization of human immunodeficiency virus type 1: genetic clades do not correspond to neutralization serotypes but partially correspond to gp120 antigenic serotypes. J.Virol. 70, 427-444 (1996).

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