



HIV-1 p24 ANTIGEN ELISA

Devised by Dr. A. Trkola, based on the method developed by Dr. John Moore

Introduction

The assay is a twin-site sandwich ELISA based on a previously published method (Ref :Moore et al., 1990, 1991) . Briefly, p24 antigen is captured from a detergent lysate of virions by a polyclonal antibody adsorbed to a solid phase. Bound p24 is detected with an alkaline phosphatase-conjugated anti-p24 monoclonal antibody and a luminescent detection system. The luminescence readout gives a broader dynamic range (2.5 logs versus 1 log with the AMPAK system) and an extended linear range which allows more accurate quantification. Furthermore, the broader dynamic range in this system makes the testing of serial dilutions of samples unnecessary in most cases. It also makes it unnecessary in most cases to re-analyze samples because their p24 content is out of linear range. This reduces the overall number of ELISA samples to be tested and minimizes handling and sample preparation times.

Antibodies

- **Coating antibody D7320, sheep anti-HIV-1- p24 gag**, affinity purified, 2mg/vial; store at +2 to 8°C. This is supplied by **Aalto Bio Reagents Ltd., Dublin, Ireland.**

[Tel : +353-1-4900685; Fax: +353-1-4900122 ; e-mail :
info@aaltobioreagents.ie]

This is a mixture of 3 sheep polyclonal antibodies raised against peptides from the HIV-1(LAV-1) sequence, then affinity purified with the respective immunogenic peptides. The amino acid sequences used are :

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

These sequences are substantially conserved between HIV-1 isolates.

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- **Secondary conjugate antibody code BC 1071-AP, alkaline phosphatase conjugate of anti-HIV-1-p24 mouse monoclonal**, 50µl/vial; store at +2 to +8°C. This is supplied by **Aalto Bio Reagents Ltd., Dublin, Ireland**. This antibody is an alkaline phosphatase conjugate of monoclonal clone EH12E1. EH12E1 is a mouse monoclonal antibody raised against 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.

- **Reagents, Buffers**

TROPIX ELISA-Light Immunoassay system: EL100CX: chemiluminescent substrate for alkaline phosphatase with enhancer (CSPD with Sapphire-II)

10 x NaHCO₃: 42g. NaHCO₃ (SIGMA Cat No S-6014)
add 500 ml dd H₂O, pH=8.5 (keep in fridge)

1 x TBS 144mM NaCl
25mM TRIS
pH 7.5

10 x TBS for 5 liters: 421g. NaCl (SIGMA Cat No S-9888)
151g. Tris-Trizma base (SIGMA Cat No T-1503)
dd H₂O add to 5 liters
pH 7.5 (use HCl to adjust)

10 x PBS for 10 liters: 800g. NaCl
20 g. KCl
144 g. Na₂HPO₄
24 g. KH₂PO₄
fill up to 8 liters with H₂O
adjust to pH 7.4 with HCl
fill to 10 liters

10 x TROPIX wash buffer : component of ELISA-Light Kit Cat No EL100CX
For 1 liter: 100 ml 10 x assay buffer concentrate
900ml ddH₂O

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Reagents, Buffers contd

0.1% Empigen in TBS: Empigen BB Detergent ~35% solution,
SIGMA Cat No 45165

0.1% Tween in PBS: Tween-20 ,SIGMA Cat No T-1379

**p24 Standard: Recombinant HIV-1 p24 from Aalto Bio Reagents,
Code AG 6054**

Dilute with 1% FCS in TBS to 100µg/ml.
Store in 100µl aliquots at -20°C.

Sheep serum: SIGMA Cat No S-7773

2% milk (for 40 plates) : blocking buffer
10g. non-fat dry milk (Carnation)
500ml 1 X TBS
Stir for one hour and filter twice through folded felt
Paper Reeve Angel (24.0 cm)

Conjugate: Dilute BC 1071-AP in 20% sheep serum,
0.05% Tween, 1 x TBS

Plates: COSTAR white opaque 96-well plates, high binding
(Cat No 3922)

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Assay

Coating p24 Plates

Day 1: Re-suspend antibody **D7320** in 1 ml dd H₂O (2mg/ml)

Add the 1ml solution of **D7320** to 400ml of 0.1M NaHCO₃, pH = 8.5 (40ml of 10 x NaHCO₃ + 359ml dd H₂O), thereby adjusting the antibody concentration to 5 µg/ml.

Stir 2 – 3 minutes

Add 100µl/well, then, stack the plates. Place a cover plate on top and wrap with saran wrap to avoid evaporation and incubate overnight at room temperature.

Note : one 2mg vial of **D7320** is sufficient to coat 40 x 96-well plates. In contrast to the previous „AMPAK“ protocol, all wells of the 96-well plate can be used for assaying. There is no difference in coating and readout between outside and central wells.

Day 2 Prepare 500ml 2% milk in 1 x TBS:

- Stir for one hour at room temperature
- Filter twice with Reeve Angel filter paper

Wash plates twice with 1 x TBS (200ml buffer per well)

Add 100ml/well of 2% milk, then stack plates, place a cover plate on top and with saran wrap to avoid evaporation and incubate for one hour at room temp

Freeze at –20°C. (Coated plates can be stored for several weeks).

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p24 ELISA Assay

- Thaw coated p24 plates.
- Discard buffer in wells and wash once with 1 x TBS.
- Discard TBS and tap plates dry.
- Prepare p24 standard as needed, dilute standard in 0.1% Empigen in TBS
- HIV-1 positive samples to be analyzed should be treated with 1% Empigen prior to assaying to inactivate the virus and linearize the proteins. (Note: The addition of the detergent is necessary. Omitting the detergent treatment or using other detergents like Tween-20 and NP40 seriously inhibits capture by **D7320**).
- Add 100µl of samples, standards and controls to the appropriate wells and incubate for 3 hours at room temperature. To avoid evaporation, blank wells are filled with 0.1% Empigen in TBS and plates are sealed with saran wrap.
- Plates are washed twice with 1x TBS, then tapped dry.
- 100µl of conjugate is added per well (Note: each lot of the secondary antibody conjugate preparation has to be individually tested for the optimal concentration to be used. The optimal dilution range of the product usually lies between 1:5000 – 1:10000).
- Incubate for one hour at room temperature, then, wash 4 times with 0.1% Tween in PBS
- Wash plates 2 times with TROPIX wash buffer and tap dry.
- Add 50 µl/well of CSPD with Sapphire Substrate (TROPIX)
- Incubate for 30 minutes at room temperature.
- Read plates in luminometer

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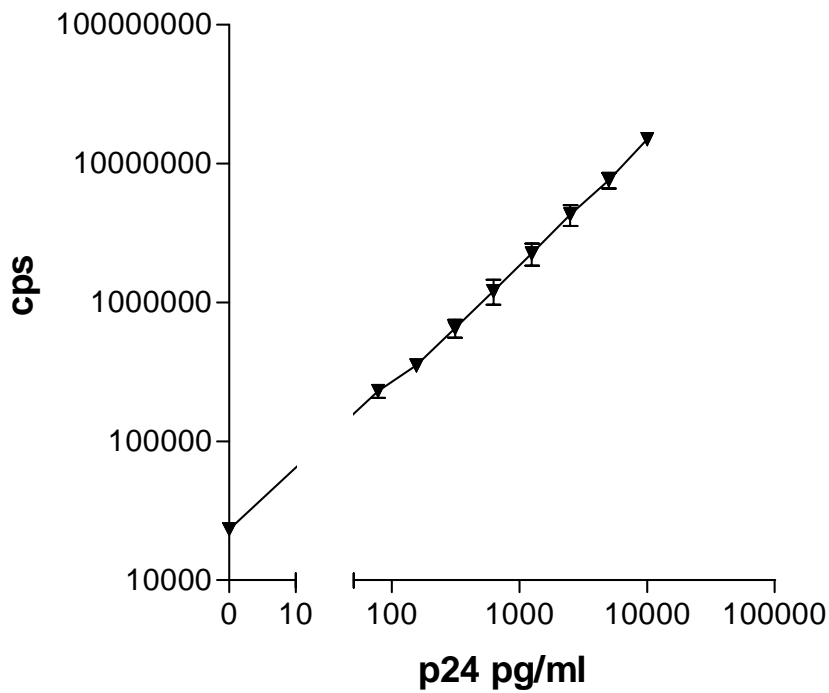
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p24 ELISA with luminescence readout



p24 ELISA with colorimetric readout

