Rapid, homogeneous and robust HIV-p24 detection assay using the AlphaLISA platform

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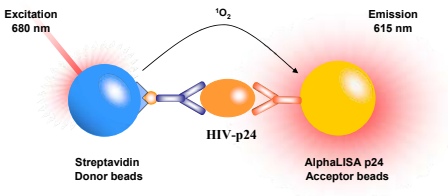
1 Introduction

The p24 protein is a major constituent of the human immunodeficiency virus (HIV) core nucleocapsid. It is highly immunogenic making it an interesting marker for the presence of viral particles. In fact, the quantitation of HIV by measuring the presence of the HIV-p24 protein is often used for different purposes, such as the diagnosis of HIV infection, disease progression, and the monitoring of the effect of therapeutic drugs.

ELISA assays are available to measure the presence of HIV-p24 in serum and cell lysates. Here, an AlphaLISA p24 detection assay has been developed in order to offer a faster and cheaper homogeneous alternative to ELISA for the detection of HIV particles. This novel sandwich assay is based on the AlphaScreen® technology where two antibodies, directed against different epitopes of p24, one biotinylated and one directly coated onto AlphaLISA Acceptor beads, simultaneously capture the HIV-p24 molecule.

2 HIV-p24 AlphaLISA Assay Scheme



The AlphaLISA platform is based on the AlphaScreen technology. It is a bead based technology that relies on the transformation of ambient oxygen into singlet oxygen by a photosynthesizer present inside the donor beads (in blue), upon irradiation at 680 nm. When a biomolecular interaction brings the Donor and Acceptor beads into close proximity (A and B interaction), the singlet oxygen will diffuse to activate a series of energy transfers inside the Acceptor beads (in yellow) that will result in the emission of light between 520 and 620 nm. In the absence of a biomolecular interaction, singlet oxygen will not reach the Acceptor beads and no signal will be emitted.

The AlphaLISA HIV-1 p24 assay is a sandwich assay where two antibodies are used to capture the HIV-p24 protein either recombinant, or present in serum samples. One generic antibody is directly conjugated onto the AlphaLISA Acceptor beads while the second antibody is biotinylated and captured using streptavidin coated Donor beads. Following interaction of the two antibodies with p24, beads are brought into close proximity and a signal is generated at 615 nm.

3 Materials

- Affinity purified anti-HIV-1-p24 antibody (Aalto Cat. No D7320)
- Anti-HIV-1 p24 AlphaLISA Acceptor beads (custom product)
- Analyte: recombinant HIV-1 p24 (Aalto Cat. No. AG6054 lot 2267)
- Biotinylated anti-HIV1-p24MAb (Aalto Cat. No. BA 1071-BIOT)
- Streptavidin donor beads: PerkinElmer (Cat No. 676002)
- Assay buffer: 25 mM Hepes, 0.1% Casein, 1 mg/mL Dextran T-500, 0.5% Triton X-100, pH 7.
- ProxiPlate-96 microplate: PerkinElmer (Cat. No. 6006290)
- OptiPlate-384 microplate: PerkinElmer (Cat. No. 6007290)
- EnVision Multilabel reader with AlphaScreen option: PerkinElmer

4 Assay Protocol

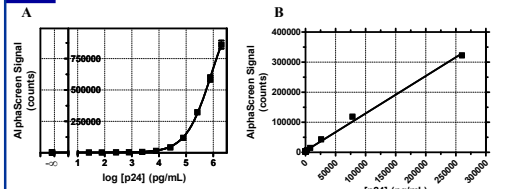
In the well of a 384 or 96-well plate add the following:

	1 µL sample	20 µL sample
1.	1 µL HIV-1-p24 dilution	20 µL HIV-1-p24 dilution
2.	29 µL biotinylated anti-HIV-1-p24 (1 nM final concentration in the well) + AlphaLISA anti-HIV-1-p24 Acceptor beads (20 µg/mL final in the well) mix	10 µL biotinylated anti-HIV-1-p24 (1 nM final concentration in the well) + AlphaLISA anti-HIV-1-p24 Acceptor beads (20 µg/mL final in the well) mix
3.	Incubate 30 min at room temperature	
4.	20 µL streptavidin Donor beads (20 µg/mL final in the well)	
5.	Incubate 60 min at room temperature, in the dark	
6.	Read using the EnVision - Alpha multilabel reader	

5 ELISA versus AlphaLISA
Assay Format Comparison

	ELISA	AlphaLISA
Homogenous	No, several wash steps	Yes
Automation	*	***
Throughput	Low	High
Sensitivity	***	***
Dynamic range	2 logs	2,5-5 logs
Microplate format	96-well plate	96- 384- 1536-well
Multiplexing	No	Not yet
Substate sizes	Small molecules to whole cells	Small molecules to large complexes
Use of polyclonal antibodies	Yes	Yes
Assay steps	more than 4	3 to 4
Total assay time	2h to Overnight	2h to Overnight (less hands on time)
Reader	ELISA readers	EnVision

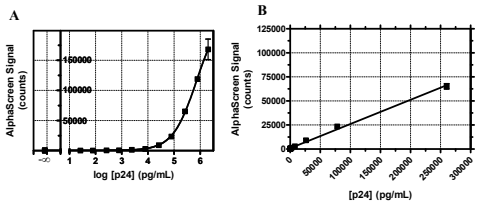
6 384-well Plate Format



In this assay 20 µL of HIV-1 p24 control was pipetted in the well of a 384-well. Binding reaction was incubated for 1h at room temperature and signal was detected using the EnVision Multi-label reader (see panel 4 for detailed description). (A) non-linear regression analysis, and (B) linear regression of the lowest concentrations.

The lower limit of detection (LDL) was calculated to be around 20 pg/well (see panel 9).

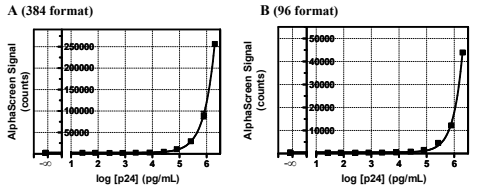
7 96-well Plate Format



In this assay 20 µL of HIV-1 p24 control was pipetted in the well of a 96-well plate. The rest of the reagents addition was the same as for the 384-well plate based assay (see panel 4). (A) Non-linear regression analysis, and (B) linear regression of the lowest concentrations.

The lower limit of detection was calculated to be around 6 pg/well (see panel 9).

8 Reduced Sample Volume



In this assay 1 µL of HIV-1 p24 control was pipetted in the well of a (A) 384- and (B) 96-well plate.

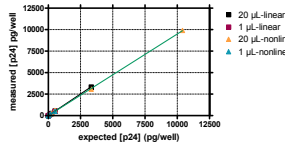
The lower limit of detection was calculated to be around 3 and 1 pg/well for 384 and 96-well plates, respectively (see panel 9). The wide dynamic range allows the use of very small sample volumes thus avoid performing serial dilutions.

9 Summary of Assay Performance

			384-Optiplate		96-Proxiplate	
			20 µL samples	1 µL samples	20 µL samples	1 µL samples
3 SD + bkg (AlphaScreen Signal)			4062	2506	671	477
LDL	nonlinear regression	pg/mL in 1 or 20 µL	575	3080	384	920
		pg/well	20	3	6	1
	linear regression	pg/mL in 1 or 20 µL	98	3047	384	2680
		pg/well	0.06	4	0.36	2
HDL	nonlinear regression	pg/mL in 1 or 20 µL	2600000	2600000	2600000	2600000
		pg/well	52000	2680	52000	2680
	linear regression	pg/mL in 1 or 20 µL	260000	780000	260000	780000
		pg/well	280	780	280	780
dynamic range	nonlinear regression		3.5 log (20-52000 pg/well)	3 log (3-2680 pg/well)	4 log (6-52000 pg/well)	3.5 log (1-2600 pg/well)
	linear regression		3.5 log (0.09-260 pg/well)	2.5 log (4-780 pg/well)	3 log (0.3-280 pg/well)	2.5 log (2-780 pg/well)

This table summarizes the assay performance in respect to these different assay formats. All formats behaved in a similar fashion with lower detection limits (LDL) between 1 and 20 pg/well, with a dynamic range around 3.5 log units.

10 Recovery in 384 Format



recovery (%) 384-Optiplate				
p24 (pg/well)	nonlinear regression analysis		linear regression analysis	
	20 µl	1 µL	20 µl	1 µL
10400	95			
2320	93		100	
520	117	95	104	98
260	97		101	
173.3	92			
165		114		105
156				102
140			106	
26		103		114
13		111		119
6.7		114		121
6.5		105		108
5.98		93		94

Recovery was measured in 384-well plate format using both 20 and 1 µL sample volumes. Experiments were performed by spiking assay buffer with known concentrations of recombinant HIV-1-p24. The measured concentrations were derived from the calibration curve performed in parallel.

Measured concentrations extrapolated from the standard curve were accurate as they matched the expected values.

11 Summary

- We have successfully developed a novel assay as an alternative to the cumbersome ELISA, using the homogeneous AlphaLISA platform.
- The AlphaLISA HIV-1-p24 assay can detect as little as 1 pg/well (300 pg/mL in 20 µL sample volume using 96-well plates) with a wide dynamic range of 3.5 log units.
- Results can be obtained with high reproducibility in one hour, without wash steps, in either 96 or 384-well plates, using down to 1 µL sample volume.
- Moreover, the assay is easy to miniaturize and automate using the Janus liquid handler in combination with the highly sensitive EnVision-Alpha reader.
- Because of its ease and speed of execution, this AlphaLISA-HIV-1-p24 assay is a major improvement over the standard ELISA procedures.
- See the following poster for more AlphaLISA assays : PST1J026