TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Caution: For Laboratory Use. A research chemical for research purposes only.

Immunodeficiency Virus type-1 p24 protein (HIV p24) (high sensitivity) Kit

Product No.: AL291 C/F

Lot specific kit information can be found at www.perkinelmer.com/COA

Material Provided

Format: AL291C: 500 assay points AL291F: 5 000 assay points

The number of assay points is based on an assay volume of 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

Product Information

Kit content: The kit contains 5 components: AlphaLISA Acceptor beads coated with an Anti-Analyte Antibody, Streptavidin-coated Donor beads, Biotinylated Anti-Analyte Antibody, lyophilized analyte and 10X AlphaLISA Immunoassay Buffer. ICD solutions are **not** provided with this kit (see page 7: ICD protocol).

Assay microplates (96-, 384- or 1536-well plates) must be purchased separately (see page 3 for more details).

Storage: Store kit in the dark at +4˚C. Store reconstituted analyte at -20°C.

Stability: This product is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions. Note: Once reconstituted, the HIV p24 analyte is stable for at least 75 days at -20°C (see page 2: Reagents and Materials).

Application: This kit is designed for the quantitative determination of HIV p24 in serum, buffered solution or cell culture medium using a homogeneous AlphaLISA assay (no wash steps).

Sensitivity: Lower Detection Limit (LDL): 1.8 pg/mL (see page 9: Assay Performance Characteristics).
A unique assay protocol has been developed for this kit (see page 5: Protocol).

Dynamic range: 1.8 – 30 000 pg/mL (see page 9: Assay Performance Characteristics).

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC_{50} and LDL were measured on an EnVision® HTS instrument using the High sensitivity protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and depending on assay conditions with no impact on LDL measurement.

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Precautions

- Only the AlphaScreen® Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous. Some analytes are from human source.
- Some analytes are present in saliva. Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Reagents and Materials

The reagents provided in the AlphaLISA kit are listed in the table below:

Kit components	**AL291C (500 assay points)**	**AL291F (5 000 assay points)**
AlphaLISA Anti-HIV p24 Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 X 1 mL @ 5 mg/mL (2 brown tubes, black caps)
Biotinylated Antibody Anti-HIV p24 stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	50 µL @ 500 nM (1 tube, black cap)	500 µL @ 500 nM (1 tube, black cap)
AlphaLISA recombinant HIV p24 (0.1 µg), lyophilized analyte *	1 tube, clear cap	1 tube, clear cap
AlphaLISA Immunoassay Buffer (10X) **	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute HIV p24 in 100 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes, if possible, or aliquoted into screw-capped polypropylene vials and stored at -20°C for further experiments. Avoid multiple freeze-thaw cycles. It has been demonstrated that reconstituted HIV p24 is stable for at least 75 days at -20°C. One vial contains an amount of HIV p24 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL291S).

** Contains 250 mM HEPES, pH 7.4, 1% Casein, 10 mg/mL Dextran-500, 5% Triton X-100 and 0.5% Proclin-300. Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).
Note: 10X buffer might be slightly yellow. However, this does not affect the assay results.

Once diluted, 1X AlphaLISA Immunoassay Buffer contains 25 mM HEPES, pH 7.4, 0.1% Casein, 1 mg/mL Dextran-500, 0.5% Triton X-100 and 0.05% Proclin-300.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	PerkinElmer Inc.	6050195
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer Inc.	-

Protocols have been optimized for 50 µL assays in white OptiPlate™-384 microplates. Other assay volumes can be used with similar protocols and identical final AlphaLISA reagent concentrations:

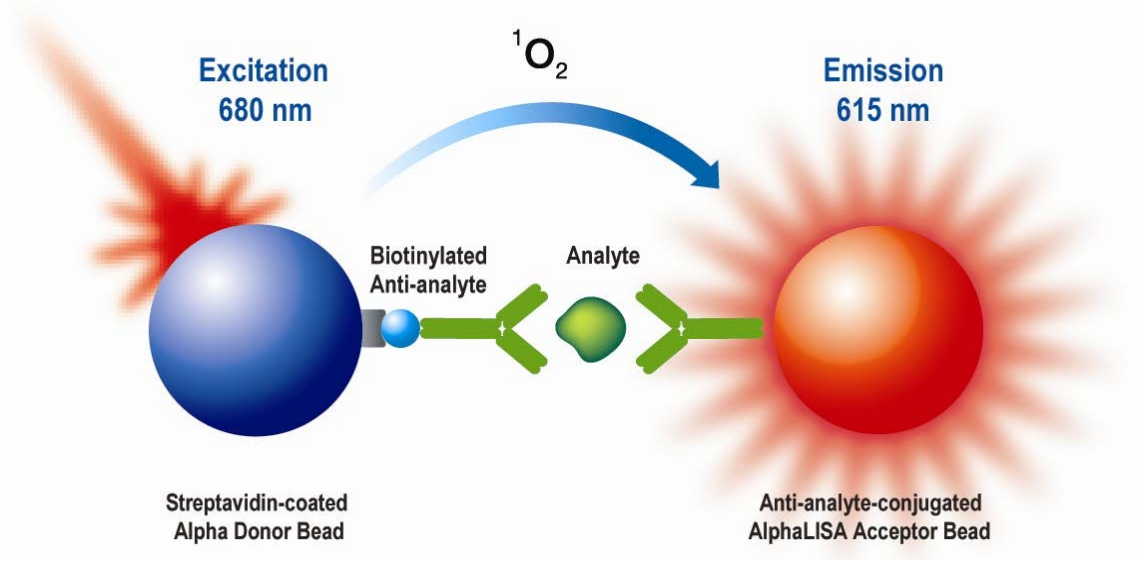
Format	# of data points	Total assay volume	Sample volume	AlphaLISA beads / Biotin Antibody MIX volume	SA-Donor beads volume	Plate recommendation
AL291C	250	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL291F	5 000	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Analyte of Interest

Immunodeficiency Virus (HIV) p24 is the 231 amino acid phosphorylated protein of the capsid forming the conical core of the virus that encapsulates the genomic RNA-nucleocapsid complex. p24 is a cleavage product of the p55 Gag polyprotein by viral proteases. HIV p24 and its 55 kDa precursor play a crucial role in the assembly, maturation, and disassembly of HIV. p24 can often be detected two weeks after infection. Subsequently, p24 antibody is produced and complexes with soluble p24 antigen, rendering it undetectable without first dissociating the antibody-antigen complex. Free antigen reappears later in the course of the illness as p24 antibody levels decline. p24 is frequently used for HIV detection in blood, serum samples, and other bodily fluids in acute HIV seroconversion, in neonatal infection, and for monitoring of responses to antiviral drug therapy.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated Donor beads while another Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (see figure below).



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Recommendations

General recommendations:

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to prewet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2 000 g, 10-15 sec). Resuspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added in the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the ALPHA option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in a similar matrix as the samples (e.g. FBS for serum samples).

Specific recommendations:

- AlphaLISA assays can be performed in cell culture medium with or without phenol red, with the following recommendations: if possible, avoid biotin-containing medium (e.g. RPMI medium) as lower counts and lower sensitivity are expected. Add at least 1% FBS or 0.1% BSA to cell culture medium.
- When analyzing serum samples, perform the standard curve in pooled human serum and use the ICD protocol (refer to page 7).

Protocol

High sensitivity protocol (2 incubation steps) – Dilution of standards in 1X AlphaLISA Immunoassay Buffer, cell culture medium or human pool serum

The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocol also includes testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.

The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

Use of four background points in triplicate (12 wells) is recommended when LDL is calculated. One background point in triplicate (3 wells) can be used when LDL is not calculated.

IMPORTANT: PLEASE READ THE RECOMMENDATIONS ABOVE BEFORE USE

Steps for Preparing Reagents

The protocol described below is for one standard curve (48 wells) and samples (452 wells). Dilution of standards can be done in 1X AlphaLISA Immunoassay Buffer, cell culture medium or human pool serum.

If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

1) Preparation of 1X AlphaLISA Immunoassay Buffer:
Add 2.5 mL of 10X AlphaLISA Immunoassay Buffer to 22.5 mL H_2O.

2) Preparation of HIV p24 analyte standard dilution (see step 6 and 7 for serum samples):
Reconstitute lyophilized HIV p24 (0.1 µg) in 100 µL H_2O.
Prepare standard dilutions as follows (change tip between each standard dilution):

Tube	Vol. of HIV p24 (µL)	Vol. of diluent (µL) *	[HIV p24] in standard curve	
			(g/mL in 5 µL)	(pg/mL in 5 µL)
A	10 µL of reconstituted HIV p24	90	1E-07	100 000
B	60 µL of tube A	140	3E-08	30 000
C	60 µL of tube B	120	1E-08	10 000
D	60 µL of tube C	140	3E-09	3 000
E	60 µL of tube D	120	1E-09	1 000
F	60 µL of tube E	140	3E-10	300
G	60 µL of tube F	120	1E-10	100
H	60 µL of tube G	140	3E-11	30
I	60 µL of tube H	120	1E-11	10
J	60 µL of tube I	140	3E-12	3
K	60 µL of tube J	120	1E-12	1
L	60 µL of tube K	140	3E-13	0.3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer, cell culture medium or pooled human serum). At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 10X AlphaLISA Anti-HIV p24 Acceptor beads + Biotinylated Antibody Anti-HIV p24 MIX (100 µg/mL / 10 nM):
Add 50 µL of 5 mg/mL AlphaLISA Anti-HIV p24 Acceptor beads and 50 µL of 500 nM Biotinylated Antibody Anti-HIV p24 to 2 400 µL of 1X AlphaLISA Immunoassay Buffer. Prepare just before use.

4) Preparation of 1.25X Streptavidin (SA) Donor beads (50 µg/mL): Keep the beads under subdued laboratory lighting.
Add 200 µL of 5 mg/mL SA-Donor beads to 19 800 µL of 1X AlphaLISA Immunoassay Buffer.

5) Samples: If applicable, dilute samples to be tested in diluent (e.g. 1X AlphaLISA Immunoassay Buffer, cell culture medium or pooled human serum).

6) Immune Complex Disruption (ICD): **step for human serum samples only**

During HIV infection, antibodies are produced to viral antigens. These specific antibodies then bind to viral antigens and form immune complexes. Bound antigen is no longer detectable. The HIV p24 AlphaLISA kit provides a protocol for the disruption of antigen/antibody complexes using a combination of low pH and heat. The samples are then neutralized and transferred to microplate wells and assayed as usual, thus allowing the previously bound antigen to be measured in serum samples.

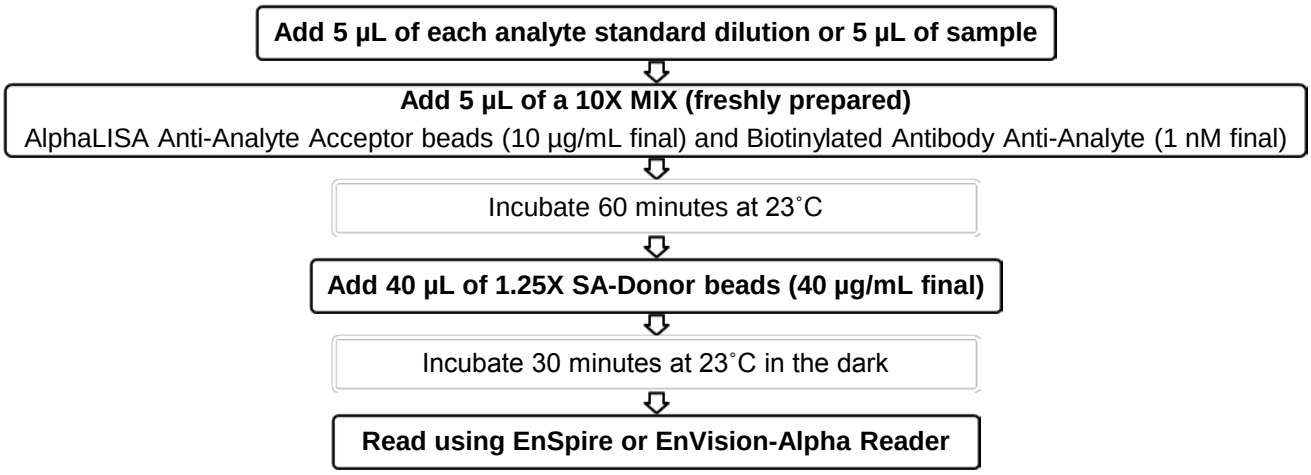
ICD solutions (not supplied with the kit):

- Glycine 1.5 M, pH 1.85 (± 0.05)
  pH is critical, calibrate carefully. Adjust pH with HCl very slowly and carefully during final adjustment.
- Tris 1.5 M, pH 11.0 (± 0.1)
- Triton X-100, 5%

7) ICD protocol:

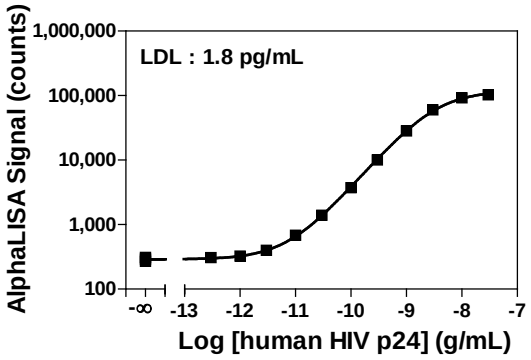
- Add 4.45 µL 5% Triton X-100 to a new microplate (96-wells). Use as many wells as there are standards (including zeros) and samples.
- Add 20 µL of samples/standards to designated wells.
- Add 20 µL Glycine using a multichannel pipettor.
  Gently mix well contents by slowly drawing up and dispensing the contents five times. Change the pipettor tips between each strip.
- Cover the plate with TopSeal-A film and incubate for 60 minutes at 37°C.
- Add 20 µL Tris Reagent using a multichannel pipettor and mix five times. Change pipettor tips between each strip.
- Incubate plate 10 to 20 minutes at room temperature (15-30°C).
- Dispense 5 µL in triplicates for each sample in a new microplate, and proceed with the standard AlphaLISA detection protocol.

8) In a 96- or 384-well microplate:

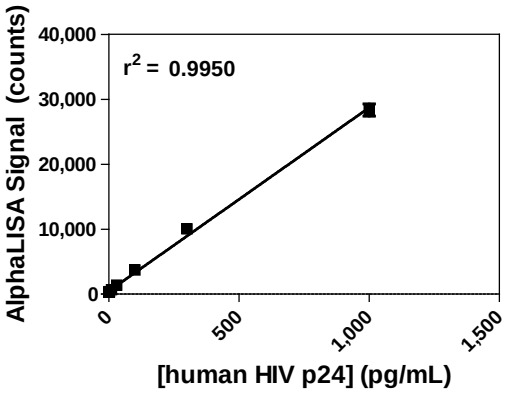


Typical results in 1X AlphaLISA Immunoassay Buffer

Log-Log scale:



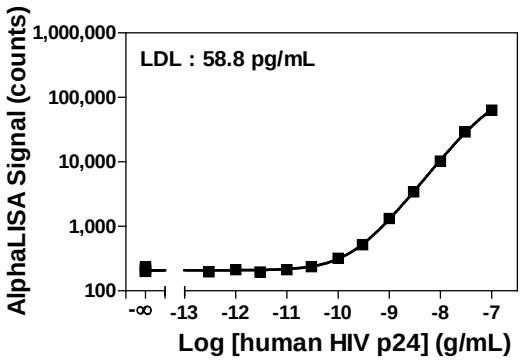
Linear-Linear scale (linear range):



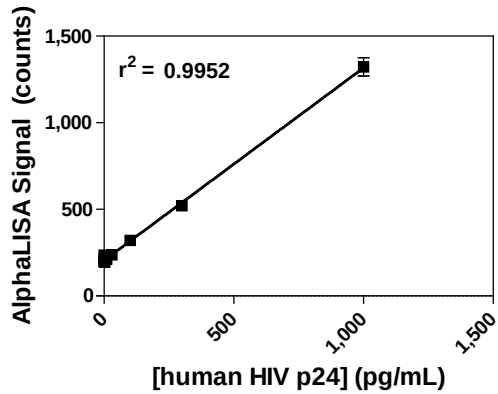
The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102.

Typical results in pooled human serum (ICD protocol)

Log-Log scale:



Linear-Linear scale (linear range):



The data was generated using a white Optiplate-384 microplate and an EnVision-Alpha Reader 2102.

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Interpreting the Data

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

Sensitivity:

The LDL was calculated as described above. This value corresponds to the lowest concentration of analyte that can be detected in a volume of 5 µL using the recommended assay conditions.

- Average LDL is 1.8 pg/mL * (using 5 µL of analyte in AlphaLISA Immunoassay Buffer) (mean of 18 independent experiments).
- Average LDL is 58.8 pg/mL (using 5 µL of analyte in pooled human serum) (mean of 6 independent experiments).

* Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10 µL of analyte in a final assay volume of 50 µL).

Dynamic range: 1.8 – 30 000 pg/mL (in AlphaLISA Immunoassay Buffer)

Assay precision:

The following assay precision data were calculated from a total of 18 assays. Two operators performed three independent assays using three different kit lots. Each assay consisted of one standard curve and three control samples of high (A), medium (B) and low (C) concentration, assayed in triplicate. The assays were performed in 384-well format using AlphaLISA Immunoassay Buffer.

- Intra-assay precision:

The intra-assay precision was determined using a total of 18 independent determinations in triplicate for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 18)
A	1 147	49.8	4.3
B	333	15.3	4.6
C	34	1.9	5.5

- Inter-assay precision:

The inter-assay precision was determined using a total of 6 independent determinations with 9 measurements for each control sample.

Sample	Mean (pg/mL)	SD (pg/mL)	% CV (n = 6)
A	1 147	72.5	6.3
B	333	17.3	5.2
C	34	2.7	7.8

Human serum experiments:

In the following experiments, the ICD protocol was used to treat the human serum samples.

- Dilutional linearity:

The dilutional linearity was determined by serial dilutions of human serum from six individuals spiked with 1 ng/mL of HIV p24. The recovery was calculated using the undiluted sample as the 100% value. The average recovery from two independent measurements is reported.

	% Recovery					
Dilution Factor	Human serum 1	Human serum 2	Human serum 3	Human serum 4	Human serum 5	Human serum 6
1	100	100	100	100	100	100
2	102	107	101	109	103	101
4	91	100	91	99	101	96
8	91	100	94	110	102	105

- Recovery:

Three known concentrations of analyte were spiked in human serum from six individuals. All samples, including non-spiked serum, were measured in the assay. Values calculated for spiked samples reflect subtraction of the endogenous (no-spike) value. The % in serum versus expected (control spike value) was calculated for each concentration. The average recovery from two independent measurements is reported.

	% Recovery					
Spike (ng/mL)	Human serum 1	Human serum 2	Human serum 3	Human serum 4	Human serum 5	Human serum 6
30	119	96	117	112	107	104
3	105	85	98	100	89	93
0.3	109	92	90	99	95	86

Specificity:

Cross-reactivity of the AlphaLISA HIV p24 Kit was tested using the following proteins at 0.03 µg/mL in AlphaLISA Immunoassay Buffer.

Protein	% Cross-reactivity	LDL (pg/mL)
p24 clade E	34	83.1
HIV-2 p26	0	-
SIV p27	0	-
p55	72	20.3

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