QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls Each lab should assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. Reproducible control values indicate proper assay performance. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. 4705

HIV-1 p24 ELISA Kit

Cat. # 4705, 96 Tests

For Quantitation of Human HIV-P24 in serum, plasma, and cell culture supernatants



For in vitro research use only (RUO), not for therapeutic or diagnostic use.

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ELISA Kit Components	Amount	Cat/Part No.
HIV-P24 **Microplate coated** with anti-Human HIV-P24	8-well strips (12)	4706-P
Wash Buffer Concentrate (20X)	25 ml	WB-4705
Standard Protein, **25 ng/vial**	2 vials	SP-4707
Detection Antibody HIV-P24, **100 ng/vial**	2 vials	4708-D
HRP-Streptavidin Conc.(450X)	0.2 ml	4709-HRP
TMB Substrate	12 ml	TM-4705
Stop Solution	8 ml	ST-4705
Assay Diluent (5X)	15 ml	AD-4705
Product Manual	1 ea	4705-M

INTENDED USE

The Human HIV-1 P24 ELISA is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human HIV P24 in serum (human HIV-P24 concentration is low in normal serum/plasma, and not be detectable in this assay), plasma and cell culture supernatants. **For in vitro research use only (RUO)**, not for therapeutic or diagnostic use.

INTRODUCTION

P24 protein is derived from the Gag protein of HIV-1 and plays an important role in viral core assembly and maturation. HIV-1 RNA, anti-HIV antibodies, and p24 antigen are viral markers which have been used as a target antigen for early detection of HIV-1 infection. Over the past two decades, HIV immunoassays have gone through first-generation (using viral lysate for IgG antibody detection), second-generation (using recombinant antigens for IgG antibody detection), third-generation (IgM and IgG antibodies detection), and fourth-generation (antibody and p24 antigen detection) immunoassay. These test kits have helped to shorten the "window period" and provide an early diagnosis for suspected HIV infected samples as compared with the third-generation immunoassays.

PRINCIPLE OF THE TEST

The Human HIV-1 P24 ELISA kit is based on the binding of HIV-P24 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of HIV-P24 present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450 nm is then measured using an ELISA microtiter well reader.

STORAGE AND STABILITY

The entire kit may be stored at **-20°C** for up to 1 year from the date of shipment. The kit may be stored at **4°C** for up to 6 months. Avoid repeated freeze- thaw cycles.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit detects Human HIV-P24. Other species not determined.

Sensitivity

The minimum detectable dose of Human HIV-P24 was determined to be **6.5 pg/ml**.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer)

Recovery

Recovery was determined by spiking various levels of Human HIVP24 into the sample types listed below.

Sample recovery (%):

- **Serum**: (108-137%)
- **Plasma** :(68-144%)
- **Cell Culture media** (74-116%)

Linearity

Sample dilution at 1:2

- **Serum: (**115-139%)
- **Plasma :**(108-139%)
- **Cell Culture media (**74-116%)

Sample dilution at 1:4

- **Serum: (124-138%)**
- **Plasma :(102-144%)**
- **Cell Culture media (72-97%)**

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, HIV-1 P24 concentrations may be determined as follows:

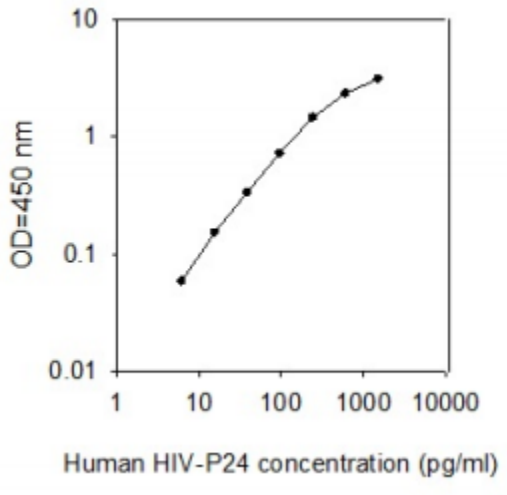
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of HIV-1 P24 (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The HIV-1 P24 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Samples producing signals higher than the 1500 pg/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Stds/samples	Mean $A_{450\ nm}$
A1, A2	**Std. A** (0 pg/ml)	0.05
B1, B2	**Std. B** (6.14 pg/ml)	0.220
C1, C2	**Std. C** (15.36 pg/ml)	0.311
D1, D2	**Std. D** (38.40 pg/ml	0.443
E1, E2	**Std. E** (96 pg/ml)	0.644
F1, F2	**Std. F** (240 pg/ml)	0.899
G1, G2	**Std. G** (600 pg/ml)	1.314
H1, H2	**Std. H** (1500 pg/ml)	1.845

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Standard Protein (2 vials of Human HIV-P24) Part No. SP-4707	1 vial is enough to run each standard in duplicate. **Preparation of standard**: Briefly spin a vial. Add 400 µl of 1x Assay Diluent into standard vial to prepare a 25 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 30 µl of the HIV-P24 standard solution from the standard vial, into a tube with 470 µl 1x Assay Diluent to prepare a 1,500 pg/ml standard solution. Pipette 300 µl 1x Assay Diluent into each tube. Use the 1,500 pg/ml standard solution to produce a dilution series. Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the zero standard (0 pg/ml).
Assay Diluent (5X) Part. No. 4705-AD , 15 ml	Dilute 5-fold with deionized or distilled water before use.
Wash Solution Concentrate (20x) Cat. No. WB-4705, 25 ml	Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Detection Antibody HIV-P24 Part No. 4708-D	2 vials of biotinylated anti-Human HIV-P24. Each vial is enough to assay half the microplate. Briefly spin the Detection Antibody vial before use. Add 100 µl of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent. Use within the working day and discard. Return concentrate to 2-8º C storage.
HRP-Streptavidin Concentrate (450x) Part No. 4709-HRP, 0.2ml	Peroxidase conjugated streptavidin in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; HRP-Streptavidin concentrate should be diluted 450-fold with 1X Assay Diluent. Use within the working day and discard. Return concentrate to 2-8º C storage. For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 9 ml 1X Assay Diluent to prepare a 450-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix Well.

Sample dilution: 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

Note: Levels of HIV-P24 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
HIV-P24 Microplate		8-well strips (12)	Coated with anti-Human HIV-P24. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
TMB Substrate	TM-4705	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	ST-4705	8 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions

PRECAUTIONS AND SAFETY INSTRUCTIONS

Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, and dispose of these samples and containers as biohazard waste.

Standards, Controls, Sample Diluent, Detection Antibody and Streptavidin-HRP contain bromo-nitro-dioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.
MSDS for TMB, sulfuric acid and BND, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Cell culture supernatants, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to two weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to room temperature (**18-25º C**) equilibration.

Use freshly diluted Standards as described on page 2. Dilute samples in Working Sample Diluent according to expected HIV-P24 concentrations

1. Bring all reagents and samples to room temperature (**18-25ºC**) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add **100 µl of each standard (see page 2) and sample** into appropriate wells. Cover wells and incubate for **2.5 hours** at room temperature with gentle shaking.
4. Aspirate and wash the wells 4 times with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. Add 100 µl **1X biotinylated antibody** (see page 2) to each well. Incubate for **1 hour** at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl **1X Streptavidin conjugate** (see page 2) to each well. Incubate for **45 minutes** at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add **100 µl TMB Substrate Reagent** to each well. Incubate for **30 minutes** at room temperature in the dark with gentle shaking. (standards and positive wells develop blue color).
10. Immediately add **50 µL Stop Solution** into each of the wells. Mix gently (blue color turns yellow). Read the absorbance at 450 nm.