HIV1 p24 ELISA Kit

HIV1 p24 ELISA Kit is an Enzyme Immunoassay kit for the quantification of HIV1 p24 in lentiviral cell culture supernatant.

Catalog number: ARG82822

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

p24 is a component of the HIV particle capsid. There are approximately 2000 molecules per virus particle, or at a molecule weight of 24 kDa, about 104 virus particles per picogram of p24. The onset of symptoms of AIDS correlates with a reduction in the number of CD4+ T cells and increased levels of virus and p24 in the blood. It is a component of the gag polyprotein.

Fourth-generation HIV immunoassays detect viral p24 protein in the blood (as well as patient antibodies against the virus). Previous generation tests relied on detecting patient antibodies alone; it takes about 3–4 weeks for the earliest antibodies to be detected. The p24 protein can be detected in patient blood as early as 2 weeks after HIV infection, further reducing the window period necessary to accurately detect the HIV status of the patient. [Provide by Wikipedia: P24 capsid protein]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for HIV1 24 has been pre-coated onto a 96 well microplate. HIV1 p24 antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti-p24 antibody is added and binds to p24 antigen captured by the first antibody. After washing away any unbound substances, a HRP Conjugate mouse anti-FITC antibody is added and incubation. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount

of p24 bound in the initial step. The color development is stopped by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of p24 in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store Standard at-20°C and store all other kit components at 4°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strip	4°C
Standard (10 µg/mL)	100 µL	-20°C
1000X p24 Antibody concentrate	20 µL	4°C
1000X HRP-conjugated Secondary Antibody concentrate	20 µl	4°C
Assay Diluent	50 mL (Ready-to-use)	4°C
Triton X-100 Solution (5%)	15 mL (Ready-to-use)	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL (Ready-to-use)	4°C (protect from light)
Stop Solution	12 mL (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Centrifuge and centrifuge tube
- Microplate shaker.
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Remember that your samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing BSL-2 organisms.
- Upon receipt, aliquot and store Standard at -20°C and store all other kit components at 4°C at all times.
- Return any unused microplate strips to the plate pouch with desiccant.
- For unknown samples, we recommend several dilutions for each sample.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.

- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. If the solution is blue before use, DO NOT USE IT.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

SAMPLE COLLECTION & STORAGE INFORMATION

1. Dilute lentiviral supernatant in culture medium. Include culture medium as a negative control.

   Note: Dilute 10 to 1000 folds for samples with infectious titer of 10^6-10^7 TU/mL. For unknown samples, we recommend several dilutions for each sample.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution, vortex to mix well.
3. Incubate for **30 minutes at 37°C.**

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) Storage the diluted 1X wash buffer at 2-8°C.
- 1X p24 Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X p24 Antibody concentrate into Assay Diluent to yield 1X p24 Antibody working solution. (E.g.: 10 µl of the MuLV core antigen Antibody concentrate (1000X) + 9.990 ml of Assay Diluent.) Do not store diluted solutions.
- 1X HRP-conjugated Secondary Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X HRP-conjugated Secondary Antibody concentrate into Assay Diluent to yield 1X HRP-conjugated Secondary Antibody working solution. (E.g.: 10 µl of the HRP-Secondary Antibody concentrate (1000X) + 9.990 ml of Assay Diluent.) Do not store diluted solutions.
- Recombinant HIV-1 p24 antigen standards:

  1. Prepare a series dilution of recombinant HIV-1 p24 antigen standards with Assay Diluent. The Assay Diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	p24 antigen standards (ng/mL)	Assay Diluent (µL)	Standards (µL)
S1	100	990	10 (10 µg/ml stock)
S2	50	500	500 of S1
S3	25	500	500 of S2
S4	12.5	500	500 of S3
S5	6.25	500	500 of S4
S6	3.125	500	500 of S5
S7	1.5625	500	500 of S6
S0	0	500	0

2. Transfer 225µL of each dilution to a microcentrifuge tube containing 25 µl of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

Note: Upon receipt, the Standard should be aliquoted and stored at ≤-20°C to avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (18- 25°C) before use, each vial should be mixed thoroughly without foaming prior to use. Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Prepare and mix all reagents thoroughly before use.
2. Add **110 µL** of **inactive sample** or **each diluted Standards** to Antibody Coated Microplate. Cover the plate and incubate **at 4°C overnight.**
3. Remove plate cover. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of 1:1000 **diluted 1X p24 Antibody working solution** to each well. Cover the plate and incubate for **1 hour** at **room temperature** on a microplate shaker.
5. **Wash** the strip wells **3 times** according to step 3 above.
6. Add **100 µL** of 1:1000 **diluted 1X HRP-conjugated Secondary Antibody working solution** to all wells, cover the plate and incubate for **1 hour at RT** on a microplate shaker.
7. **Warm TMB substrate solution to RT** before next wash step. Aspirate each well and **wash** as step 3. Proceed immediately to the next step.
8. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at **450nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each dilution of Standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. There are approximately 2000 molecules of p24 per Lentiviral Particle (LP), therefore,

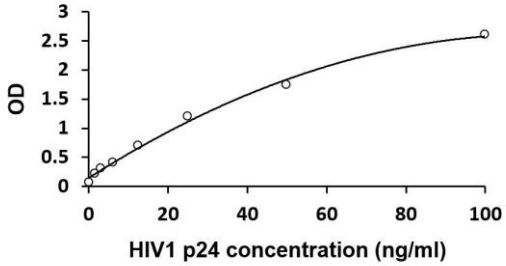
   1 LP contains: $(2000 \times 24 \times 10^3) / (6 \times 10^{23})$ g of p24 or

1 ng p24 = 1.25 x 10^7 LPs.

7. For a reasonably packaged lentivirus vector, 1 TU is about 100 to 1000 LP, therefore: 10^6 TU/mL = 10^{8-9} LP/mL = 8 to 80 ng/mL
8. *The calculated result is the lentivirus physical titer, p24 core protein level, and it is NOT the infectious titer (TU/mL). When the infectious titer is determined, the results vary among different target cell lines or transduction methods.*

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the HIV1 p24 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1 ng/mL