HIV1 p24 ELISA Kit

(Rapid One-Step)

ARG83111 HIV1 p24 ELISA Kit (Rapid One-Step) is an Enzyme Immunoassay kit for the quantification of HIV1 p24 in all lentiviral biological samples.

Catalog number: ARG83111

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

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

HIV1 p24 ELISA Kit (Rapid One-Step) 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; an anti- HIV-1 p24 antibody conjugate is added and binds to p24 antigen, 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

Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 X 12 strips	4°C
Standard S7-S0 (1000, 333.33, 111.11, 37.04, 12.35, 4.12, 1.37, 0 ng/mL)	8 vails (ready to use)	4°C
Sample Diluent Buffer	50 mL (ready to use)	4°C
10X Wash Buffer	50 mL	4°C
Lysis Buffer	6 mL (ready to use)	4°C
Antibody Conjugate	6 mL (ready to use)	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
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- All reagents must be mixed without foaming and briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer or Diluent Buffer warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- All reagents must be mixed without foaming before use.
- Change pipette tips between the addition of different reagent or samples.
- 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.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C. Mix well before use.

SAMPLE PREPARATION

- Dilution of crude virus samples and filtered samples for 50-fold dilution
- Chromatographic sample dilution for 200-fold dilution.

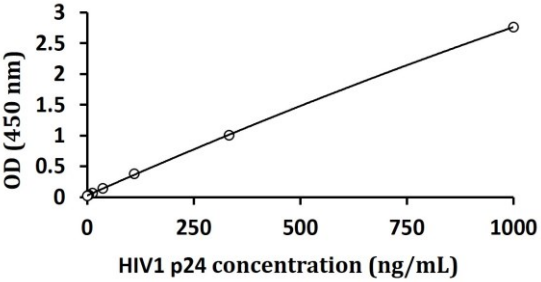
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use.

1. Add **50 µL** Lysis Buffer to each wells.
2. Add **10 µL** of the **Standard, Samples** and **Blank** to the Antibody Coated microplate.
3. Add **50 µL** of **Antibody Conjugate** to each wells.
4. Cover the plate and Incubate for **30 minutes** at **Room Temperature**.
5. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the wells for 30 sec before remove. 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.
6. Add **100 µL** of **TMB Substrate** to each well. Cover and incubate for **5-15 minutes** at **room temperature** in the dark.
7. Immediately Add **100 µL** of **Stop Solution** to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 3 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. To obtain more accurate results, more dilution points can be used when generating standard curves. 4 Parameter Logistics is the preferred method for the result calculation. Other data reduction functions may give slightly different results.
3. 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)
4. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.