For Research Use Only Not for Diagnostic Use

Rapid-Lenti HIV-1 p24 ELISA Kit (Single Wash)

Catalog# XB-4000

Introduction

Principle of the Assay

Microtitration wells coated with murine anti-HIV-1 p24 capture antibody are exposed to test specimens, which may contain HIV-1 p24 reactive determinants. A mixture of biotinylated anti-HIV-1 p24 antibodies and streptavidin HRP conjugate are then added forming a complex (HIV-1 p24/antibodies/conjugate) that binds to the wells. After an incubation period, unbound components in the test sample are washed away. Specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of HIV-1 p24 reactive determinants present in a sample.

The assay can be used to monitor HIV-1 in cell culture or to determine the viral titer of lentiviral samples and can be completed in as little at 90 minutes.

Kit Presentation

Materials Supplied
The reagents supplied in this pack are for research use only.

1	Coated microwell strips. Plastic microtitration wells coated with anti-HIV-1 p24 murine monoclonal antibody in foil pouch with desiccant.	1 plate (96 wells)
2	Positive HIV-1 p24 Control (10ng/ml)	0.1 ml
3	Lysis Buffer	6 ml
4	Detector Antibody. Anti-HIV-1 p24 conjugated to biotin.	6ml
5	Conjugate. Streptavidin conjugated to horseradish peroxidase enzyme containing 0.01% Bromonitrodioxane as preservative.	6 ml
6	Wash Buffer (20X concentrated). Tris buffered saline pH 7.8-8.0, containing 0.05% Tween 20. Must be diluted before use.	30 ml
7	Substrate solution. Tetramethylbenzidine.	12 ml
8	Stop Solution. 1 N H_2SO_4	12 ml

Additional Requirements for Manual Processing

- Disposable tip micropipettes to deliver volumes of 5 µl, 10 µl, 25 µl, 100 µl, and 200 µl (multichannel pipette preferred for dispensing reagents into microtiter plates).
- Distilled or deionized water.
- 37 (±1)˚C incubator.
- Clean, disposable plastic/glass test tubes, approximate capacities 5 ml and 10 ml.
- Clean volumetric laboratory glassware consisting of at least 15 ml and 100 ml beakers, 1 L graduated cylinder, 1 ml, 5 ml, and 10 ml glass pipettes.
- Absorbent paper towels.
- Automatic microtitration plate washer or laboratory wash bottle.

Automatic, or Semi-automatic Processing
The HIV-1 p24 Assay may be used with a variety of automatic or semi-automatic processors/liquid handling systems. It is essential that any such system is qualified, before it is used routinely, by demonstrating that the HIV-1 p24 Assay results obtained using the automatic processor are equivalent to those obtained for the same specimens using the manual test method. Subsequently, the automatic processor should be periodically re-qualified.

Storage and Stability

All reagents should be stored at 2-8°C and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Secure open foil pouch using zip top before storage.

The working strength Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash Buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

Indications of Deterioration
The HIV-1 p24 Assay may be considered to have deteriorated if:

1. The kit fails to meet the required criteria for a valid test (see Interpretation of Results).
2. Reagents becoming visibly cloudy or develop precipitate. *Note: Concentrated Wash Buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37˚C.*
3. The Substrate Solution turns dark blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Warnings and Precautions

Safety

1. The reagents supplied in this kit are for ***research use only.***
2. Caution: All blood products should be treated as potentially infectious.
   Essential precautions are summarized as follows:
   *Do not pipette by mouth.
   *Wear disposable gloves during all specimen and assay manipulations.
   *Avoid use of sharp or pointed liquid handling devices, which may puncture skin.
   *Do not smoke, eat, or drink in the laboratory working area.
   *Avoid splashing of liquid specimens and reagents and the formation of aerosols.
   *Wash hands thoroughly on completion of a manipulation.
   *The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
3. The HIV-1 p24 kits contain reagent systems, which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
4. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with great care and wear suitable protective clothing and eye/face protection. In case of contact with skin or eyes, immediately flush the affected area with plenty of water. For eye damage, seek medical attention immediately.

Procedural

1. This kit should be used in strict accordance with the instructions in the Package Insert.

2. Do not use HIV-1 p24 Assay kits after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents. Always use fresh pipettes tips when drawing from stock reagent bottles.
4. Always use clean, preferably disposable, glassware for all reagent preparation.
5. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
6. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
7. Always keep the upper surface of the microtitration strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
8. Do not allow the wells to completely dry during an assay.
9. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
10. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with HIV-1 p24 Assay by demonstration of equivalence to the manual processing methods.
11. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
12. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Collection and Storage

HIV-1 p24 Assay is intended for use with tissue culture supernatants. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

Rinse Cycle

Efficient rinsing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. The HIV-1 p24 Assay utilizes one standard six-rinse cycle. Automatic plate washers may be used provided they meet the following criteria:

1. All wells are completely aspirated.
2. All wells are filled to the rim (350 µl) during the rinse cycle.
3. Wash Buffer is dispensed at a good flow rate.
4. The microtitration plate washer must be well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures must be followed diligently.

For the rinse cycle the machine should be set to six consecutive washes. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual Wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with Wash Buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat steps 1 and 3, five additional times.
5. Invert the microtitration plate and tap firmly on absorbent paper towels.

Preparation for the Assay

Kit Positive Control (10ng/ml)

Prepare working strength Positive Control by diluting 30 µl of Positive Control into 470 µl (1:16.6 dilution) of uninoculated tissue culture media. This will give a final concentration of 300 pg/ml as shown in Table 1 after addition of Detector Antibody/Streptavidin-HRP Mix (Assay Procedure, Step 6).

Detector Antibody/Streptavidin-HRP Mixture

Premix the Detector Antibody and Streptavidin-HRP in a 1:1 ratio. Each well will require 80 µl of solution (40 µl Detector Antibody + 40 µl Streptavidin-HRP). To create enough solution for the entire plate we recommend mixing 5 mL of Detector Antibody into 5 mL of Streptavidin-HRP. Mixing of Detector Antibody and Streptavidin-HRP should be completed immediately before addition to designated wells.

Wash Buffer

Prepare working strength Wash buffer by diluting 1-part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs. Each row of 8 wells may be adequately washed with 20 ml of working strength buffer.

Quantitative Assay Procedure

To test quantitatively, a standard curve should be prepared using tissue culture media as the diluent as shown in the table below. Each standard dilution in addition to inoculated tissue culture control should be run in duplicate.

Table 1:

P24 Quantitative Standard Curve Generation			
Tubes	**Addition to Tube**	**Media (µl)**	**p24 (pg/mL)**
1	30 µl of 10 ng/mL p24	470	300
2	250 µl of Tube 1	250	150
3	250 µl of Tube 2	250	75
4	250 µl of Tube 3	250	37.5
5	250 µl of Tube 4	250	18.8
6	250 µl of Tube 5	250	9.4
7	250 µl of Tube 6	250	4.7
8	0	250	0

Assay Procedure

1. Allow all reagents to reach room temperature (18 25˚C).
2. The diluted positive control (300 pg/ml) and uninoculated cell culture media (for use as a negative control/reagent blank) should be tested at least in duplicate in every assay.
3. Select sufficient microtitration well strips to accommodate all test specimens, controls and reagent blank/negative control. Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross reference system molded into the plastic frame.
4. Dispense 20 µl of lysis buffer to each well.
5. Dispense 100 µl of each control and specimen into appropriate wells. **Note: All controls and samples should be tested in duplicate.**
6. Dispense 80 µl of Detector Antibody/Streptavidin-HRP mixture (see Prep. Assay Section, #2) into each well and incubate at 37(±1)˚C for 60 (±5) minutes. Plate shaking at 400 rpm is recommended but not necessary.
7. Aspirate the contents from the wells and wash the microtitration plate as described in the Rinse Cycle section (6 washes.

8. Without delay, dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18 25˚C) protected from direct sunlight, for 30 (±2) minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well including the reagent blank/negative control. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
10. Immediately after adding the Stop Solution, read the absorbance values at 450 nm using a microtitration plate reader **blanked on the negative control/reagent blank well.**

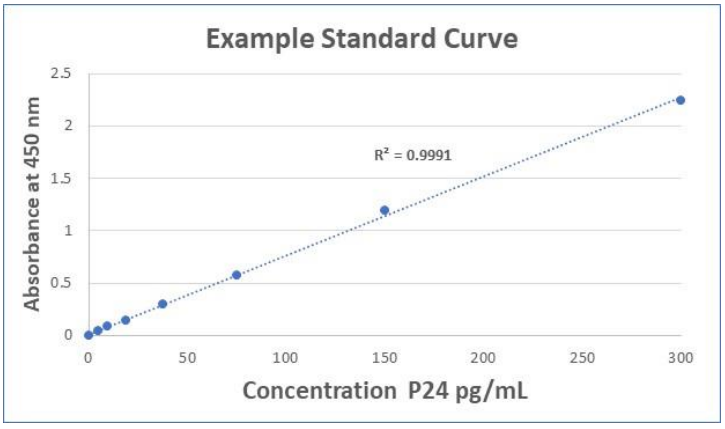
Interpretation of Results

Quantitative Analysis

Manual Method: The calibration curve can be constructed manually on linear graph paper by plotting the mean absorbance for each standard on the y-axis versus the concentration of the standard (value printed on vial) on the x-axis. Connect the points to produce a point-to-point curve. Do not force the line to be linear. The concentration of the specimens can be found directly from the standard curve.

Table 2: Example Data at 450nm.

Standards	450nm absorbance
Standard 1 (0 pg/mL)	0.0
Standard 2 (4.7 pg/mL)	0.04
Standard 3 (9.4 pg/mL)	0.08
Standard 4 (18.8 pg/mL)	0.14
Standard 5 (37.5 pg/mL)	0.30
Standard 6 (75 pg/mL)	0.57
Standard 7 (150 pg/mL)	1.19
Standard 8 (300 pg/mL)	2.24



Note: This standard curve is only an example and should not be used to generate any results.

Computer Assisted Method: Computer assisted data reduction may be used to create the standard curve. Software providing a point-to-point curve fitting routine provides acceptable results.

Assay Validation
The HIV-1 p24 assay should be considered valid if:

The negative control/reagent blank should be ≤0.10 after blanking.
The 150 pg/ml control should be ≥ 0.60.

Determining Lentivirus Titer (TU)

The lentivirus titer can be calculated from the values determined in the assay. The following calculations are based on approximately 2000 molecules of p24 in one physical particle of lentivirus (LP). Therefore, one LP contains 8 x 10^{-5} pg of p24 (2000 x molecular weight/Avogadro) or 1 ng of p24 equals 1.25 x 10^{7} LPs. A well packaged lentivirus vector will have 1 TU for every 100 -1000 LPs. A supernatants titer of 10^{6} TU/ml will have 10^{8-9} LP/ml or 8 to 80 ng/ml. A supernatants titer of 10^{7} will have 80 to 800 ng/ml.

Procedure note for samples with HIV-1 p24 assay values greater than the highest standard:

Many tissue culture samples will have p24 values greater than the highest standard. In order to obtain accurate results for samples with HIV-1 p24 assay values greater than the highest standard it is necessary to dilute and re-test the sample. Diluting the specimen 10-fold is recommended. For example: Make a 10-fold dilution by adding 0.1 ml of the initial specimen to 0.90 ml of tissue culture medium. Mix thoroughly and repeat the assay according to the Assay Procedure. Multiply the results by 10 to determine the correct HIV-1 p24 assay values in the sample.

Limitations of Use

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably.
2. Samples with very high HIV-1 p24 assay values levels may exhibit the prozone effect. For this assay, antigen levels must be greater than 50,000 pg/ml before the assay gives erroneous results of less than 300 pg/ml.
3. The assay cannot be used to quantitate samples with HIV-1 p24 assay values greater than the highest standard without further serial dilution of the samples. See the Interpretation of Results section for directions on testing such samples.
4. The performance characteristics have not been established for any matrices other than tissue culture media.

Performance Characteristics

Analytical Sensitivity: To determine the sensitivity of the assay, the 0 standard was assayed 20 times. The minimal detectable level was calculated by adding two standard deviations to the mean absorbance for the 0 standard. The minimal detectable level is 1.8 pg/ml.

Linearity: Four strongly reactive samples were serially two-fold diluted and run on the assay. The values obtained were compared to the expected values by standard linear regression. The r values obtained ranged from 0.993 to 1.0.

Precision: Four samples with different levels of activity were assayed ten times each on three different assays. The results are summarized in the table on the following page.

Precision Data:

		Sample 1	Sample 2	Sample 3	Sample 4
Assay 1 (n = 10)	**Mean (pg/mL)**	206.5	102	50.4	27.5
	SD	8.76	5.51	3.8	2.4
	CV	4.24%	5.4%	7.55%	8.7%
Assay 2 (n = 10)	**Mean (pg/mL)**	203.6	75.8	42.7	25.9
	SD	10.6	5.11	3.4	2.27
	CV	5.2%	6.75%	7.96%	8.7%
Assay 3 (n = 10)	**Mean (pg/mL)**	209.8	86.9	46.5	26.8
	SD	8.144	6.0	2.15	2.4
	CV	3.88%	6.92%	4.6%	8.9%
Inter-Assay (n = 30)	**Mean (pg/mL)**	209.9	88.2	46.6	26.8
	SD	9.3	12.2	4.4	2.4
	CV	4.4%	13.8%	9.5%	8.8%

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