Version 29-04/24

User's Manual

Lentivirus Titer Kit, HIV-1 p24 ELISA

REF **DEIA3571**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For quantitative detection of HIV-1 p24 Antigen in tissue culture supernatants for lentiviral particles titration.

Principles of Testing

Microtitration wells coated with anti-HIV-1 P24 capture antibody, are exposed to test specimens, which may contain HIV-1 p24 reactive determinants. The HIV-1 p24 antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured antigen is then reacted with a biotinylated HIV-1 p24 detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. Following a wash cycle, 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 p24 in cell culture or to determine the viral titer of lentiviral samples.

Reagents And Materials Provided

1. HIV-1 p24 Antibody Coated 96-well Plate in foil pouch with desiccant: 1
2. Recombinant HIV-1 p24 Standard (10ng/ml): 0.1 mL
3. Biotinylated HIV-1 p24 Detection Antibody: 12 mL
4. Streptavidin Conjugated Horseradish Peroxidase: 12 mL
5. Lysis Buffer: 5 mL
6. 20× Plate Wash Buffer: 60 mL
7. Substrate Solution (TMB): 12 mL
8. Stop Solution (1N HCl): 12 mL
9. Plate Sealer: 3

Materials Required But Not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Absorbent paper towels.
6. 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.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.

9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard infectious waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.
13. 1% sodium hypochlorite as disinfectant. May be prepared from household bleach.
14. Blank cell culture media (without FBS)

Storage

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.

Specimen Collection And Preparation

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.

Reagent Preparation

1. Kit Standard Stock 10 ng/mL

Prepare standard 1 by diluting 20µL of standard stock into 980 µL (1:50 dilution) of blank tissue culture media (without FBS). This will give a final concentration of 200 pg/mL at shown in the table below.

Tubes	Addition to Tube	Media (µL)	p24 (pg/mL)
1	20 µl of 10 ng/mL p24	980	200
2	500 µl of Tube 1	500	100
3	500 µl of Tube 2	500	50
4	500 µl of Tube 3	500	25
5	500 µl of Tube 4	500	12.5
6	0	500	0

2. 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.

Assay Procedure

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 sixrinse 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 2 and 3, five times.
5. Invert the microtitration plate and tap firmly on absorbent paper towels.

Procedure

1. Allow all reagents to reach room temperature (18-25°C).
2. The diluted standards and uninoculated cell culture media (for use as a negative control) 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. 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 200 µL of each standard, control and specimen into appropriate wells. Note: All standards, controls and samples should be tested in duplicate. Depending on the titer of your lentivirus or specimen samples, dilution may be needed. The recommended dilution range for lenti-viral sample is from 1:500 to 1:5000 in blank cell culture media. If the sample titer is not known, make serial dilution to titrate the sample.
6. Incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.
7. Discard liquid in the plate and tap the plate firmly on paper towels or other absorbent materials to remove residual liquid in the wells. Do NOT let the wells completely dry.
8. Pipette 100 µL of detector antibody into each well and incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.
9. Discard liquid in the plate and tap the plate firmly on paper towels or other absorbent materials to remove residual liquid in the wells. Do NOT let the wells completely dry.
10. Pipette 100 µL of Streptavidin HRP conjugate into each well and incubate for 30 min at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

11. Aspirate the conjugate from the wells and wash the microtitration plate as described in the Rinse Cycle section.
12. 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) and protected from direct sunlight for 20-25 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well including the reagent blank. 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.
14. Immediately after adding the Stop solution, read the absorbance values at 450 nm using a microtitration plate reader blanked on the negative control well.

Note

1. If plate shaker is not available, shake the plate by hand for 10 seconds to mix the solution in the well after adding the Lysis Buffer and Protein Standard/Samples, and increase the incubation time to 2 hours.
2. Without shaking during plate incubation period, the signal will be lower than expected, however, it has no significant influence on data analysis.
3. To ensure accurate results and avoid crosscontamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays.
4. All reagents should be added to the plate in the same order.
5. Protect Substrate Solution from light.
6. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).

Quality Control

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

The negative control should be ≤ 0.10

The 100 pg/ml control should be ≥ 0.60

Calculation

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).

1 PP contains 8×10^{-5} pg of p24 (derived from $2000 \times 24 \times 10^{3}$Da/ (6×10^{23}) g).

1 $\times 10^{4}$ PP of lentivirus for every pg of p24 antigen.

About 100 physical particles (PP) contain 1 transducing unit (TU). Therefore 10^{7} TU/mL = 10^{9}PP/mL = 1×10^{5} pg/mL).

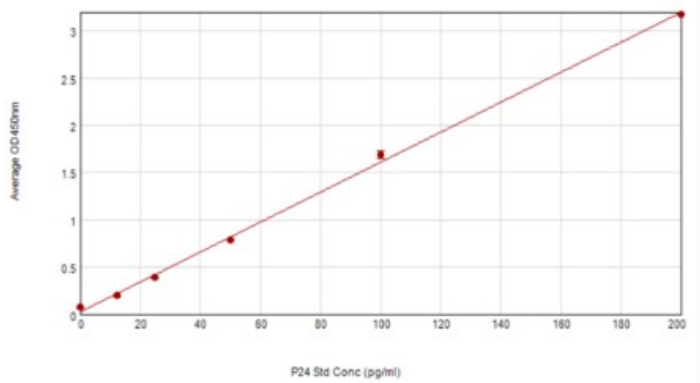
If your samples are diluted, Multiply the results by the dilution factor to determine the correct HIV-1 p24 assay values in the samples.

Typical Standard Curve

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 xaxis. 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.

Standards	450 nm absorbance
Standard 1 (0 pg/mL)	0.069
Standard 2 (12.5 pg/mL)	0.195
Standard 3 (25 pg/mL)	0.384
Standard 4 (50 pg/mL)	0.788
Standard 5 (100 pg/mL)	1.689
Standard 6 (200 pg/mL)	3.172



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. A linear regression or a 4-parameter logistic (4-PL) model providing a point to point curve fitting provides acceptable results.

Precision

Three samples with different levels of p24 were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Sample	CV% in Assay 1	CV% in Assay 2	CV% in Assay 3	Average Intra-assay CV%
Sample 1 (n=10)	2.93	2.58	4.81	3.44
Sample 2 (n=10)	3.82	2.93	3.88	3.55
Sample 3 (n=10)	3.97	2.70	2.19	2.96

Sample	Mean (pg/mL) in assay 1	Mean (pg/mL) in assay 2	Mean (pg/mL) in assay 3	Average (pg/mL)	SD	Inter-assay CV%
Sample 1 (n=10)	23.80	23.28	23.82	23.55	0.38	1.61
Sample 2 (n=10)	108.60	99.92	115.65	107.78	11.13	10.33
Sample 3 (n=10)	176.67	151.73	163.96	157.84	8.65	5.48

Sensitivity

1.6pg/mL

Linearity

To assess the linearity of the assay, samples spiked with HIV-1 P24 were diluted with Blank Media to produce samples with values within the dynamic range of the assay.

Sample	% of Expected
1:2 spiked sample	94.4
1:4 spiked sample	103.9
1:8 spiked sample	93.2

Recovery

The recovery of HIV-1 P24 spiked to three different-levels of the assay range in diluted samples was evaluated.

Sample Type	Average % Recovery
Diluted lenti-viral sample	96

Precautions

1. The reagents supplied in this kit are for Research use only.
2. Caution: All blood products should be treated as potentially infectious.
3. Essential precautions can be 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 work 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 Bio safety Level 2.
4. 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.
5. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with 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 eyes, obtain medical attention.
6. This kit should be used in strict accordance with the instructions in the Package Insert.

7. Do not use HIV-1 p24 Assay kits after the expiration date printed on the outer carton label.
8. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
9. Always use clean, preferably disposable, glassware for all reagent preparation.
10. 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.
11. 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.
12. 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.
13. Do not allow the wells to completely dry during an assay.
14. 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.
15. 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.
16. 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.
17. 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.

Limitations

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 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 200 pg/mL.
3. The assay cannot be used to quantitate samples with HIV1 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.