For Research Use Only  Not for Diagnostic Use

ORIGENE

One Wash™ Lentivirus Titer Kit, HIV-1 p24 ELISA
Catalog No. TR30038  TR30038P5

Principle of the Assay

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.

Kit Presentation

Materials Supplied

The reagents supplied in this pack are for Research Use Only.

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 p24 Antibody Coated 96-well Plate in foil pouch with desiccant</td>
<td>1</td>
</tr>
<tr>
<td>Recombinant HIV-1 p24 Standard (10ng/ml)</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Biotinylated HIV-1 p24 Detection Antibody</td>
<td>12 mL</td>
</tr>
<tr>
<td>Streptavidin Conjugated Horseradish Peroxidase</td>
<td>12 mL</td>
</tr>
<tr>
<td>Lysis Buffer</td>
<td>5 mL</td>
</tr>
<tr>
<td>20x Plate Wash Buffer</td>
<td>60 mL</td>
</tr>
<tr>
<td>Substrate Solution (TMB)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution (1N HCl)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3</td>
</tr>
</tbody>
</table>

Additional Requirements for Manual Processing

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.
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)

TECHNICAL HINTS

- 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.
- Without shaking during plate incubation period, the signal will be lower than expected, however, it has no significant influence on data analysis.
- To ensure accurate results and avoid cross-contamination, 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.
- All reagents should be added to the plate in the same order.
- Protect Substrate Solution from light.
- 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).

Related OriGene Products

1. Lenti-ORF, Plasmid and Ready-to-use Particles
2. Lenti-shRNA, Plasmid and Ready-to-use Particles
3. Lentiviral Packaging Kits, high efficiency
4. Lenti Concentrator, concentrate lentivirus in 2hrs

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 requalified.

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 Precaution

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 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.
>Wear disposable gloves during all specimen and assay manipulations.
Do not interchange vial caps or stoppers either within or between reagents from kits with different lot numbers. Do not dispose or decontaminate 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 2 and 3, five times.
5. Invert the microtitation plate and tap firmly on absorbent paper towels.

**Preparation for the Assay**

1. **Kit Standard Stock 10 ng/mL**

Prepare standard 1 by diluting 20 µL 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 as shown in Table 1

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.

### Quantitative Assay Procedure

To test quantitatively, a standard curve should be prepared using blank cell culture media (without FBS) 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

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Addition to Tube</th>
<th>Media (µL)</th>
<th>p24 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 µL of 10 ng/mL p24</td>
<td>980</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>500 µL of Tube 1</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>500 µL of Tube 2</td>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>500 µL of Tube 3</td>
<td>500</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>500 µL of Tube 4</td>
<td>500</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

**Assay 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 microtitation 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 lentiviral 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 microtitation 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 microtitation plate reader blanked on the negative control 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 specimen can be found directly from the standard curve

#### Table 2. Example Data at 450nm.

<table>
<thead>
<tr>
<th>Standards</th>
<th>450 nm absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 (0 pg/mL)</td>
<td>0.069</td>
</tr>
<tr>
<td>Standard 2 (12.5 pg/mL)</td>
<td>0.195</td>
</tr>
<tr>
<td>Standard 3 (25 pg/mL)</td>
<td>0.384</td>
</tr>
<tr>
<td>Standard 4 (50 pg/mL)</td>
<td>0.788</td>
</tr>
<tr>
<td>Standard 5 (100 pg/mL)</td>
<td>1.689</td>
</tr>
<tr>
<td>Standard 6 (200 pg/mL)</td>
<td>3.172</td>
</tr>
</tbody>
</table>

**Typical HIV-1 p24 Antigen ELISA Kit Standard Curve**

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.
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.

Assay validation
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

Performance Characteristics

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted lenti-viral sample</td>
<td>96</td>
</tr>
</tbody>
</table>

2. 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2 spiked sample</td>
<td>94.4</td>
</tr>
<tr>
<td>1:4 spiked sample</td>
<td>103.9</td>
</tr>
<tr>
<td>1:8 spiked sample</td>
<td>93.2</td>
</tr>
</tbody>
</table>

3. Sensitivity: 1.6 pg/mL

4. 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.

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).

- 1 PP contains $8 \times 10^{-5}$ pg of p24 (derived from $2000 \times 24 \times 10^3$ Da / $(6 \times 10^{23})$ g).
- 1 x $10^4$ 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 = $1 \times 10^5$ 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.

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 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 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.

Contact Information:
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Web: www.origene.com

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Assay Flowchart

1. Add 20 µL of LB, 200 µL of Stds and Diluted Samples
   Incubate 1 hours at RT with Shaking

   Discard liquid, tap the plate on paper towels

2. Add 100 µL of Detection Antibody
   Incubate 1 hour at RT with Shaking

   Discard liquid, tap the plate on paper towels

3. Add 100 µL of SA-HRP
   Incubate 30 minutes at RT with Shaking

   Wash 6 times

4. Add 100 µL of TMB Substrate
   Incubate 20-25 minutes at RT

5. Add 100 µL of Stop Solution

   Read in Plate Reader