ZeptoMetrix®

# HIV-1 p24 Antigen ELISA 2.0

Catalog Number:
0801002

## INTENDED USE

The RETRO-TEK HIV-1 p24 Antigen ELISA 2.0 is an enzyme linked immunoassay used to detect Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen in cell culture media. It can be used to monitor the purification and biochemical behavior of HIV-1 or determine the titer of HIV-1-based lentiviral samples. Since the amino acid sequence of p24 is well conserved among the various HIV-1 isolates, this assay detects p24 from HIV-1 clades A-F. There is no cross reactivity to Human Immunodeficiency Virus Type-2 (HIV-2), Simian Immunodeficiency Virus (SIV) or Human T-Cell Leukemia Virus Types I and II (HTLV I and II) p24 or any *gag* gene product.

**The RETRO-TEK HIV-1 p24 Antigen ELISA 2.0 is supplied for research use only. Not for use in diagnostic procedures.**

## PRINCIPLE OF THE TEST

Microplate wells are coated with a monoclonal antibody specific for the p24 *gag* gene product of HIV-1. HIV-1 p24 antigen in the sample is specifically captured onto the immobilized antibody during sample incubation. The captured antigen is then reacted with a human anti-HIV-1 antibody conjugated to horseradish peroxidase (HRP). Following the subsequent addition of substrate, color develops as the HRP enzyme reacts. The resultant optical density is proportional to the amount of HIV-1 p24 antigen present in the sample. The absorbance values of a set of standard dilutions are then plotted. The amount of p24 is determined by interpolation from a point-to-point plot or from a linear regression analysis of the standard curve.

## REAGENTS

**Materials Supplied:**

- **HIV-1 p24 Antibody Coated Microplate for 96 determinations,1 plate:** 12x8 well strips. Wells coated with a murine monoclonal antibody to HIV-1 p24.
- **HIV-1 p24 Detector Antibody, 12 ml**: Contains HRP (Horseradish peroxidase)-conjugated human antibody to HIV-1 purified from HIV-1 human source material.
- **HIV-1 p24 Antigen Standard, 0.5 ml:** Contains detergent-disrupted, heat-inactivated viral antigen from HIV-1 $_{IIIB}$, goat serum, Triton X-100®, and sodium azide.
- **Lysing Buffer, 5 ml:** Triton X-100® in PBS and 2-chloroacetamide.
- **10X Plate Wash Buffer, 125 ml:** Contains PBS, Tween 20®, and 2-chloroacetamide.
- **Substrate (12 ml):** Contains Tetramethyl Benzidine (TMB)
- **Stop Solution, 12 ml:** Contains hydrochloric acid (HCl).
- **Plate Sealers:** 10
- **Resealable Plastic Bag**

® Triton X-100 is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.
Tween 20 is a registered trademark of Imperial Chemical Industries.



| | | | |
|---|---|---|---|
| REF | Catalog Number | | Temperature Limitation |
| LOT | Batch Code | | Expiration Date |
| RUO | For Research Use Only | | Biological Risk |

**Storage:**

Store all kit reagents at 2° - 8°C. DO NOT FREEZE.
When stored properly the kit is stable until the date indicated on the box label.

**Materials required but not supplied:**

- **Disposable gloves**
- **Validated adjustable micropipettes, single and multichannel**
- **Test tubes and racks for preparing specimen and control dilutions**
- **Graduated cylinders and assorted beakers**
- **Validated automatic microplate washer or manual vacuum aspiration equipment**
- **Validated incubator for 37°C ±1°C**
- **Validated microplate reader**
- **Timer**
- **1% sodium hypochlorite as disinfectant. May be prepared from household bleach**
- **Distilled or deionized water**

## PRECAUTIONS

**FOR RESEARCH USE ONLY. NOT FOR *in vitro* DIAGNOSTIC USE.**

- Prior to performing the assay, carefully read all instructions.
- Use universal precautions when handling kit components and test specimens.
- To avoid cross-contamination, use separate pipet tips for each specimen.
- When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
- HIV-1 p24 Antigen Standard contains sodium azide as a preservative. Sodium azide may react with lead or copper pipes to form explosive metal azides. Flush pipes with large quantities of water upon disposal to prevent azide buildup in drains.
- Stop Solution contains hydrochloric acid, which may cause severe burns. In case of contact with eyes or skin, rinse immediately with water and seek medical assistance. Wear protective clothing and eye wears.
- Human source material used in the manufacture of the HIV-1 Detector Antibody has been tested and found negative for Hepatitis B surface antigen. The viral lysate used to prepare the HIV-1 p24 Antigen Standard has been inactivated by chemical disruption and heating. Handle these reagents as if capable of transmitting infectious agents.
- Although the p24 antigen is well conserved some HIV-1 isolates and recombinant clones may show slight reactive differences compared to the HIV-1 p24 Antigen Standard included this test kit.

## PREPARATION OF REAGENTS

- **Bring all reagents to room temperature before use.**
- **Plate Wash Buffer:** Dilute 10X Plate Wash Buffer 1:10 in distilled or deionized water prior to use. 1X Plate Wash Buffer may be stored at 2°-8°C for up to 1 week. Additional bottles of 10X Plate Wash Buffer (Catalog #: 0801060) may be ordered
- **Plate Washing:** Set dispense volume of plate washer or other dispensing equipment to at least 350µl or as high as possible without overflowing the microplate wells. This will ensure complete washing of the well and prevent potential signal background caused by reagent splashing that may occur during pipetting or plate sealer removal.
- **Test Preparation:** Label test tubes to be used for the preparation of standards and specimens. Label each strip on its end tab to identify the strips should they become detached from the plate frame during the assay. If the entire 96 well plate will not be used, remove surplus strips from the plate frame. Place surplus strips and desiccant into the Resealable Plastic Bag, seal and store at 2° - 8°C.
- **HIV-1 p24 Antigen Standard**: Prepare a series of six standards from the HIV-1 p24 Antigen Standard. Use the dilution scheme in Table 1. Any diluted HIV-1 p24 Antigen Standard remaining after the completion of the assay should be discarded.

**Table 1**

**Preparation of HIV-1 p24 Antigen Standard**

| Standard Number | Concentration of HIV-1 p24 (pg/ml) | HIV-1 p24 Antigen Standard (µl) | Assay Diluent (µl) |
|---|---|---|---|
| 1 | 125.0 | 50 | 950 |
| 2 | 62.5 | 500 of #1 | 500 |
| 3 | 31.3 | 500 of #2 | 500 |
| 4 | 15.6 | 500 of #3 | 500 |
| 5 | 7.8 | 500 of #4 | 500 |
| 6 | 3.9 | 500 of #5 | 500 |
| 7 | 0 | 0 | 500 |

**Standards do not require treatment with Lysing Buffer.**

- **Test Samples:** Treat samples in a test tube by pipetting 50 µl of Lysing Buffer into 450 µl sample and mix well. Alternatively, other volumes may be used as long as sample is disrupted with 1/10 volume of Lysing Buffer. For unknown samples it is recommended to prepare a set of 10-fold dilutions to ensure one of the dilutions falls within the standard curve. Dilutions can be made with either Assay Diluent or cell culture media. Treated samples may be stored long term at -20°C or colder if necessary.

## TEST PROCEDURE

**Step 1:** Prewash each well of the microplate or the selected number of strips 3 times with 350 µl of 1X Plate Wash Buffer and aspirate. Prewashing removes the microplate stabilizer and is necessary to achieve uniform and optimal reactivity. Thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells. **Do not allow washed plates to dry completely prior to sample addition. Drying will adversely affect test results.**

**Step 2:** Configure two strips with prepared standards as shown below. Leave one well of the microplate empty during the assay. This well is used for a substrate blank. Pipette 200 µl of standards #1-6 into duplicate wells and #7 (0 pg/ml) into triplicate wells.

| | 1 | 2 |
|---|---|---|
| **A** | 125 pg/ml | 125 pg/ml |
| **B** | 62.5 pg/ml | 62.5 pg/ml |
| **C** | 31.3 pg/ml | 31.3 pg/ml |
| **D** | 15.6 pg/ml | 15.6 pg/ml |
| **E** | 7.8 pg/ml | 7.8 pg/ml |
| **F** | 3.9 pg/ml | 3.9 pg/ml |
| **G** | 0 pg/ml | 0 pg/ml |
| **H** | Substrate Blank | 0 pg/ml |

**Step 3:** Pipette 200 µl of each test sample, prepared as described in the preparation of reagents section, into duplicate wells.

**Step 4:** Cover microplate with a plate sealer and incubate for at least 1.5 hours or overnight (up to 24 hours) at 37°C ± 1°C. Shorter incubation time may cause microplate edge effects. Longer incubation time increases sensitivity.

**Step 5:** Aspirate and wash plate 6 times as described in Step 1.

**Step 6:** Pipette 100 µl of HIV-1 p24 Detector Antibody into each well, except the substrate blank. Cover the microplate with a plate sealer and incubate for 1 hour at 37°C ± 1°C.

**Step 7:** Aspirate and wash plate 6 times as described in Step 1.

**Step 8:** Pipette 100 µl of Substrate into all wells and incubate uncovered for 30 minutes at room temperature (18°- 25°C). A blue color will develop in wells containing viral antigen.

**Step 9:** Stop the reaction by pipetting 100 µl of Stop Solution into each well. A color change from blue to yellow will result.

**Step 10:** Within 15 minutes, read the optical density of each well at 450 nm using a microplate reader.

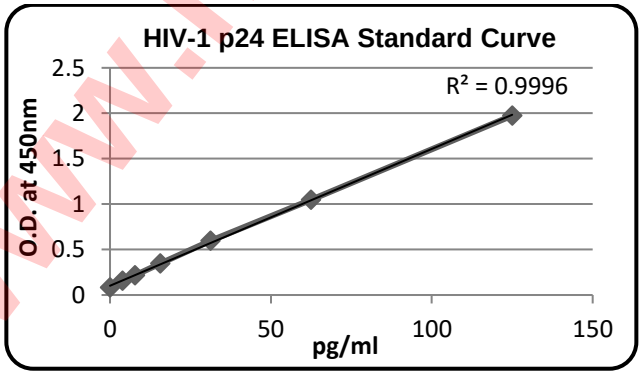
## TEST VALIDITY

- The optical density of the 0.0 pg/ml standard should not be above 0.120. If two or more wells are above 0.120, then the test is invalid.
- The optical density values of the 125 pg/ml p24 standard should be greater than 1.100 otherwise the test is invalid.
- The mean absorbance reading of a test sample must be below the 125 pg/ml value, otherwise the sample must be retested at a higher dilution to be within the linear range of the assay. If the mean absorbance reading of the test sample is below the 3.9 pg/ml value, then the p24 concentration is below the limit of quantitation of the assay.

**TYPICAL STANDARD CURVE**

| HIV-1 Antigen Concentration | Avg. OD at 450nm * |
|---|---|
| 125 pg/ml | 1.974 |
| 62.5 pg/ml | 1.049 |
| 31.3 pg/ml | 0.596 |
| 15.6 pg/ml | 0.345 |
| 7.8 pg/ml | 0.217 |
| 3.9 pg/ml | 0.155 |
| 0 pg/ml | 0.082 |

* Overnight assay values will be higher than the 1.5 hour results. Standard curves may vary as a result of incubation time and temperature, laboratory temperature, etc.



## CALCULATION

**Determination of the Cutoff Value:**

A predetermined factor of 0.030 is added to the mean of the 0.0 pg/ml sample optical value. This is an estimate of the limit of detection of the assay. The value is a general guideline to determine the cutoff value. Alternatively, more accurate results may be obtained by adding at least two standard deviations to the mean optical density from a set of known negative specimen samples to establish the statistical cutoff value. Samples with absorbance values greater than or equal to the cutoff value but below the 3.9 pg/ml mean absorbance value are considered qualitatively positive.

## To Quantitate Levels of HIV-1 p24:

Calculate the mean absorbance for each p24 standard and test sample. Using linear graph paper or computer graphing software, plot the concentration of HIV-1 p24 Antigen Standard (pg/ml) on the X-axis versus the mean optical densities for each standard on the Y-axis. Then determine the concentration of HIV-1 p24 antigen in specimens by interpolation or linear regression analysis from the standard curve. Be sure to correct for all dilutions, including the 1.1 dilution made during the addition of Lysing Buffer. Test sample absorbance values must be within the standard curve to accurately quantify.

## Determination of viral titer (TU/ml):

There are approximately 2000 molecules of p24 per physical particle (PP) of lentivirus: $(2 \times 10^3) \times (24 \times 10^3$ Da of p24 per PP), $48 \times 10^6$ / Avogadro = $(48 \times 10^6) / (6 \times 10^{23}) = 8 \times 10^{-17}$g of p24 per PP, approximately 1 PP per $1 \times 10^{-16}$g of p24, $1 \times 10^4$ PP per pg of p24. A reasonably well packaged, VSV-G pseudotyped lentiviral vector will have an infectivity index in the range of 1 TU per 1000 physical particles (PP) to 1 TU per 100 PP (or less). Thus, the range is approximately 10 to 100 TU/pg of p24.

## PRECISION DATA

| | | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|---|
| **Intra-Assay 1 (n = 16)** | **Mean (pg/ml)** | 117.80 | 22.96 | 3.63 |
| | **SD** | 5.48 | 1.00 | 0.56 |
| | **CV** | 4.66% | 4.36% | 15.52% |
| **Intra-Assay 2 (n = 16)** | **Mean (pg/ml)** | 117.85 | 22.20 | 3.49 |
| | **SD** | 2.67 | 1.66 | 0.44 |
| | **CV** | 2.26% | 7.46% | 12.73% |
| **Intra-Assay 3 (n = 16)** | **Mean (pg/ml)** | 118.86 | 23.84 | 3.52 |
| | **SD** | 6.20 | 1.18 | 0.39 |
| | **CV** | 5.22% | 4.95% | 11.03% |
| **Inter-Assay (n = 48)** | **Mean (pg/ml)** | 118.17 | 23.00 | 3.55 |
| | **SD** | 4.92 | 1.45 | 0.46 |
| | **CV** | 4.17% | 6.28% | 12.88% |

## REFERENCES

Theophilus S. Vijaykumar, Avindra Nath, and Ashok Chauhan, “Chloroquine mediated molecular tuning of astrocytes for enhanced permissiveness to HIV infection,” *Virology* 381, no. 1 (November 10, 2008): 1-5.

Cherie T Ng et al., “Passive neutralizing antibody controls SHIV viremia and enhances B cell responses in infant macaques,” *Nat Med* 16, no. 10 (October 2010): 1117-1119.

Manuel A. F. V. Gonçalves et al., “Rapid and Sensitive Lentivirus Vector-Based Conditional Gene Expression Assay to Monitor and Quantify Cell Fusion Activity,” *PLoS ONE* 5, no. 6 (June 3, 2010): e10954.

## PROCEDURAL FLOW CHART

**PREPARE REAGENTS**

↓

**WASH PLATE**

↓

**PIPET SPECIMENS, STANDARDS AND CONTROLS**

↓

**INCUBATE 1.5 HOURS TO OVERNIGHT AT 37º ± 1ºC**

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**WASH PLATE**

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**PIPET DETECTOR ANTIBODY**

↓

**INCUBATE 1 HOUR AT 37º ± 1ºC**

↓

**WASH PLATE**

↓

**PIPET SUBSTRATE**

↓

**INCUBATE 30 MINUTES AT ROOM TEMPERATURE**

↓

**ADD STOP SOLUTION AND READ AT 450 NM**