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User's Manual

Simian Immunodeficiency Virus p27 Antigen ELISA Kit

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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.

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PRODUCT INFORMATION

Intended Use

The SIV p27 Antigen ELISA KIT is used to detect Simian Immunodeficiency Virus (SIV) p27 antigen in research specimens, including cell culture media.

General Description

Simian immunodeficiency viruses (SIVs) are retroviruses able to infect at least 45 species of African non-human primates. Virus strains from two of these primate species, SIVsmm in sooty mangabeys and SIVcpz in chimpanzees, are believed to have crossed the species barrier into humans, resulting in HIV-2 and HIV-1, respectively. The most likely route of transmission of HIV-1 to humans involves contact with the blood of chimps that are often hunted for bushmeat in Africa.

Principles of Testing

Microwells are coated with a monoclonal antibody specific for the p27 gag gene product of SIV. Viral antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured antigen is then reacted with a high-titer polyclonal anti-SIV antibody conjugated to biotin. Following a subsequent incubation with streptavidin peroxidase, color develops as the bound enzyme reacts with the substrate. Resultant optical density is proportional to the amount of SIV p27 antigen present in the specimen.

Reagents And Materials Provided

1. SIV p27 Antibody Coated Microplate for 96 determinations, 1 plate: 12 x 8 well strips.
2. SIV p27 Detector Antibody, 1 vial, lyophilized: Contains biotin-labelled polyclonal antibody to SIV, milk, Tween, normal human serum and PBS.
3. Detector Antibody Diluent, 25 ml: Contains ProClin 300.
4. SIV p27 Antigen Standard, 0.5 ml: Contains detergent-disrupted, heat-inactivated viral antigen, goat serum, TritonX-100 and sodium azide.
5. Lysing Buffer, 5 ml: Triton X-100 in PBS and 2-chloroacetamide.
6. Streptavidin peroxidase, 0.3 ml: Contains streptavidin conjugated to horseradish peroxidase, PBS, goat serum, and 2-chloroacetamide.
7. Assay Diluent, 100 ml: Contains goat serum, PBS, Triton X-100 and 2-chloroacetamide.
8. 10X Plate Wash Buffer, 125 ml: Contains PBS, Tween 20 and 2-chloroacetamide.
9. Substrate, 0.5 ml: Contains tetramethylbenzidine (TMB) and dimethyl sulfoxide.
10. Substrate Buffer, 50 ml: Contains citrate/acetate buffer, hydrogen peroxide and 2-chloroacetamide.
11. Stop Solution, 12 ml: Proprietary formulation.
12. Plate Sealers, 10.
13. Resealable Plastic Bag.

Materials Required But Not Supplied

1. Disposable gloves
2. Validated adjustable micropipettes, single and multichannel
3. Test tubes and racks for preparing specimen and control dilutions
4. Graduated cylinders and assorted beakers
5. Validated automatic microplate washer or manual vacuum aspiration equipment
6. Validated incubator for 37°C ± 1°C
7. Validated microplate reader
8. Timer
9. 1% sodium hypochlorite as disinfectant. May be prepared from household bleach
10. Distilled or deionized water

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.

Reagent Preparation

SIV p27 Detector Antibody:

Add 11.0 ml of Detector Antibody Diluent to the bottle of SIV p27 Detector Antibody. Mix or rock gently for at least 1 hour. Reconstituted reagent can be stored for one week at 2-8°C. Alternatively, the reconstituted SIV p27 Detector Antibody can be divided into aliquots and frozen at -20°C.

SIV p27 Antigen Standard:

Prepare a series of six standards from the SIV p27 Antigen Standard. Use the dilution scheme in the table below. Any diluted SIV p27 Antigen Standard remaining after the completion of the assay should be disinfected and discarded. Do not save diluted reagent.

Preparation of SIV p27 Antigen Standard

Standard Number	Concentration of SIV p27 (pg/ml)	SIV p27 Antigen Standard (µl)	Assay Diluent (µl)
1	2000.0	50	950
2	1000.0	500 of #1	500
3	500.0	500 of #2	500
4	250.0	500 of #3	500
5	125.0	500 of #4	500
6	62.5	500 of #5	500
7	0	0	500

Streptavidin peroxidase and Substrate:

To prepare the Streptavidin peroxidase Working Solution and the Substrate Working Solution, use the dilution schemes in the table below. Use Substrate Working Solution within 15 minutes of its preparation. Any diluted Substrate Working Solution and Streptavidin peroxidase Working Solution remaining after the completion of the assay must be discarded.

Preparation of Streptavidin peroxidase Working Solution and Substrate Working Solution

Number of Strips Used	Streptavidin peroxidase Working Solution		Substrate Working Solution	
	Streptavidin peroxidase(µl)	Assay Diluent (ml)	Substrate(µl)	Substrate Buffer (ml)
3	40	4.0	40	4.0
6	70	7.0	70	7.0
9	100	10.0	100	10.0
12	120	12.0	120	12.0

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 may be ordered.

Assay Procedure

Allow all reagents to reach room temperature before use. 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 is not 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.

1. Treat specimens in a test tube by pipetting 50 µl of Lysing Buffer into 450 µl specimen and mix well.
2. Wash each well of the microplate or the selected number of strips 6 times with 300µl of 1X Plate Wash Buffer and aspirate. 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 plates to dry completely prior to sample addition. Drying will adversely affect test results.
3. Leave one well of the microtiter plate empty during the assay. This well is used for a substrate blank.
4. Pipet 200 µl of standards #1-6 into duplicate wells.
5. Pipet 200 µl of each specimen, as prepared in Step 1, into duplicate wells.
6. Cover microplate with a plate sealer and incubate as follows, either.

a. Overnight at 37°C ± 1°C or b. 2 hours at 37°C ± 1°C

7. Aspirate and wash plate as described in Step 2.
8. Pipet 100 µl of reconstituted SIV p27 Detector Antibody into each well, except the substrate blank. Cover the microplate with a sealer and incubate for 1 hour at 37°C ± 1°C.
9. Aspirate and wash plate as described in Step 2.
10. Pipet 100 µl of the Streptavidin Peroxidase Working Solution into each well except the substrate blank. Cover the microplate with a sealer and incubate for 30 minutes at 37°C ± 1°C.
11. Aspirate and wash plate as described in Step 2.
12. Pipet 100 µl of Substrate Working Solution 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.
13. Stop the reaction by pipetting 100 µl of Stop Solution into each well. A color change from blue to yellow will result.
14. Within 15 minutes, read the optical density of each well at 450 nm using a microplate reader.

Calculation

TEST VALIDITY:

Determine the mean optical density values for each standard and specimen. For the test to be valid, it must meet the following criteria:

1) The mean optical density of the 0 pg/ml standard and the substrate blank must be less than 0.200.
2) The mean optical density of the 1000 pg/ml standard must be greater than or equal to 0.500.

Cut-off Determination for a Qualitative Assay:

1) Determine the mean optical density of the media or normal plasma control wells. Add a factor of 0.05.
2) Alternatively, run a series of normal specimens and determine cut-off as the mean plus five standard deviations.

To Quantitate Levels of SIV p27:

Using linear graph paper, plot the concentration of SIV p27 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 SIV p27 antigen in specimens by interpolation from the standard curve. Alternatively, the level of SIV p27 may be calculated by computer using a point-to-point algorithm. Be sure to correct for all dilutions, including the 1 to 1.1 dilution made during the addition of Lysing Buffer.

Typical Standard Curve

Typical Standard Curve

SIV p27 Antigen Concentration	Optical Density at 450 nm
2000.0 pg/ml	1.247
1000.0 pg/ml	0.755
500.0 pg/ml	0.469
250.0 pg/ml	0.303
125.0 pg/ml	0.208
62.5 pg/ml	0.161
0.0 pg/ml	0.108

Precautions

1. Prior to performing the assay, carefully read all instructions.
2. Use universal precautions when handling kit components and test specimens.*
3. To avoid cross-contamination, use separate pipet tips for each specimen.
4. Disposal: When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
5. Human source material used in the manufacture of the SIV Detector Antibody has been tested and found negative for Hepatitis B surface antigen. The viral lysate used to prepare the SIV-p27 Antigen Standard has been inactivated by chemical disruption and heating. Handle these reagents as if capable of transmitting

infectious agents.

6. The SIV p27 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.
7. 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 eyewear.