



SIMIAN IMMUNODEFICIENCY VIRUS

(IgG Antibody)

ELISA 96 Test Kit

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NOT SUITABLE FOR HUMAN USE
FOR RESEARCH USE ONLY
READ ENTIRE INSERT PRIOR TO TESTING

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1. INTENDED USE

The VRL SIV ELISA kit (SIV E96, Cat#: E-080121-096) is a qualitative test designed for detection of IgG antibody in serum or plasma against Simian Immunodeficiency Virus (SIV). The SIV ELISA kit is for research non-human primate only and is not suitable for human use.

This insert provides investigators with guidelines for the detection of SIV IgG antibody in nonhuman primates using the SIV E96 Kit.

8. TEST RESULTS AND INTERPRETATION

8.1. Validation: The test result will be valid when the substrate blank must be $OD < 0.2$; negative control must be negative; the positive control has an average $OD \geq 1.0$. If these criteria are not met, the test is not valid and must be repeated.

8.2. Cut-off value: $COV = \text{average OD of 3 cut-off control wells}$

8.3. Positive: $OD \text{ of specimen} \geq COV$

8.4. Negative: $OD \text{ of specimen} < 0.8 \times COV$

8.5. Indeterminate: $0.8 \times COV \leq OD \text{ of specimen} < COV$

Any indeterminate specimen is suggested to be retested or another confirmatory procedure be performed. Samples can be submitted to VRL Laboratories for confirmatory testing.

7. TEST PROCEDURE

Allow all reagents to reach room temperature before use. Microplate strips in a frame are packed with desiccant in an aluminum bag. 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 strips from the plate frame. Place strips and desiccant into the press-seal bag, close bag carefully to ensure airtight conditions and store at 2-8°C.

- 7.1. Sample dilution (1:50)** Place testing serum or plasma (EDTA, citrate, heparin) and provided serum control tubes (Vial #6, 7 and 8) on the test tube rack. Pipet 490 µl of sample diluent (0.1% BSA) per well in the deep well sample dilution plate. Add 10 µl each of control serum and testing sample into its assigned well and mix well. Note: Change tips between specimens to avoid cross contamination.
- 7.2. Adding specimen** Set up one blank, two negative control wells, two positive control wells, and three cut-off control wells in each individual test run. Add 100 µl of each diluted testing sample or control serum into each well and then seal the plate with a plate sealer. Incubate at 37°C for 1 hour.
- 7.3. Washing** After incubation, remove and discard the plate sealer. Aspirate and wash plate 4 times with wash buffer at low speed in the ELISA plate washer. Fill each well with 315 µl wash buffer during each wash. After washing step, thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells.
- 7.4. Secondary antibody conjugate** Dilute provided secondary antibody conjugate 1:2500 in sample diluent by adding 5 µl of HRP conjugate stock (vial #5) into 12.5 ml of sample diluent and mix well. Pipet 100 µl of diluted conjugate into each well, except the substrate blank. Cover the plate with a plate sealer and incubate at 37°C for 1 hour.
- 7.5. Washing** Aspirate and wash plate as described in step 7.3.
- 7.6. Color development** Pipet 100 µl of HRP substrate solution (Vial #9, read-to-use TMB) into all wells. Incubate uncovered at room temperature (18-25°C) for 5 minutes in the dark. A blue color will develop in wells containing SIV antibody.
- 7.7 Stop reaction** Stop the reaction by pipetting 100 µl of Stop Solution (Vial #10) into each well and agitate to mix. A color change from blue to yellow will result.
- 7.8. Reading** Within 15 minutes, read the optical density (OD) of each well at 450 nm against substrate blank, reference wave length at 630 nm.

2. PRINCIPLE OF THE TEST

The SIV E96 is based on the principle of indirect enzyme linked immunoassay. Microwells are coated with purified SIV antigen. After adding testing sample into the wells, any SIV antibody present in the specimen will specifically bind to the coated SIV antigen. After removal of unbound material, anti-human IgG conjugated to an enzyme (horseradish peroxidase) is allowed to react with the immuno-complex. After removal of extra conjugate by washing, an appropriate substrate (TMB) is added to have color development. Lastly, the test plate is read in a spectrophotometer to determine if SIV antibody is present in sera.

3. MATERIALS

3.1. Materials supplied

Each SIV E96 kit provides the following materials:

- #1 SIV Antigen coated microplate:** 12x8 well strips on 1 frame for 96 determinations.
- #2 Powdered phosphate buffer (PBS):** sufficient for 1 liter 1x PBS (medium colorless bottle).
- #3 Tween-20:** 2.0 ml 50% Tween-20 (orange top vial).
- #4 Sample diluent:** 3 ml 10% BSA (small colorless bottle).
- #5 Conjugate:** 10 µl goat anti-human IgG conjugated to horseradish peroxidase (colorless top vial).
- #6 PC serum:** 30 µl SIV positive control (violet top vial).
- #7 Calibrator:** 30 µl SIV cut-off control (red top vial).
- #8 NC serum:** 30 µl control serum negative for anti-SIV antibody (green top vial).
- #9 HRP Substrate:** 12 ml ready-to-use tetramethylbenzidine(TMB, amber bottle).
- #10 Stop Solution:** 15 ml ready-to-use proprietary formulation (medium colorless bottle).

3.2. Materials Required but Not Supplied

- Disposable gloves
- Disposable reagent reservoirs
- Deep well serum dilution plates
- Vortex mixer
- Validated microplate reader
- Validated microplate washer
- Validated adjustable micropipettes, single (2, 10, 100 and 1000µl) and multichannel (50-300 µl) and tips
- Validated incubator for 37°C
- Distilled water (dH₂O) or deionized water
- Timer

4. PRECAUTIONS

NOTE: THIS KIT IS FOR RESEARCH USE ONLY

- Prior to performing the assay, carefully read all instructions.
- The viral lysate antigen has been inactivated by chemical disruption and heating. Good laboratory practice, however, dictates that all materials be handled in accordance with practices employed in a bio-hazardous laboratory.
- Control sera provide in this kit have been heat inactivated.
- The components of this kit are offered as a unit. Do not use for purposes other than stated herein.
- Do not reuse reagents or mix with reagents of other kits.
- The animal and material collected from the animal must be considered to be potentially infectious. Handle these materials as hazardous and dispose accordingly.
- To avoid cross-contamination, use separate pipette tips for each specimen.
- Disposal: When testing potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
- Stop Solution contains 2M sulfuric 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.

5. STORAGE OF KITS

Store kits at 4-8°C. At this temperature, shelf life is at least 12 months. An expiration date is provided with each kit when stored at 4-8°C. After opening, microplate strips are stable for 4 weeks at 2-8°C in closed aluminum bag with desiccant.

6. REAGENT PREPARATION

6.1. Reconstitute powdered PBS (0.1M PBS)

Add contents of PBS vial (medium colorless bottle #2) to 1.0 liter distilled water (dH₂O), mix well; Keep 0.1M PBS (pH 7.2~7.4) at 4°C and it can be stored for up to 2 months.

6.2. Prepare plate wash buffer (0.05%PBST)

Add 1 ml of 50% Tween 20 (Vial #3) into 1000 ml 1xPBS to make 0.05% PBST wash buffer. Wash buffer may be stored at 2-8°C for up to 1 week.

6.3. Prepare sample diluent (0.1%BSA in PBST)

Take 1 ml of sample diluent concentrate (10% BSA in Vial #4) into 99 ml of wash buffer (1xPBST) to make 0.1 % sample diluent.