



**Simian T-cell Lymphotropic Virus  
(STLV-1)  
ELISA 96 Test Kit  
(IgG Antibody)**

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

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## 1. INTENDED USE

The VRL STLV ELISA kit (STLV E96A, Cat#: E-080118-096A) is a qualitative test designed for detection of IgG antibody in serum or plasma against Simian T-cell lymphotropic/leukemia virus type 1 (STLV-1). The STLV ELISA kit is for research use only and is not suitable for human use.

This insert provides investigators with guidelines for the detection of STLV IgG antibody in nonhuman primates using the STLV E96A Kit.

## 2. PRINCIPLE OF THE TEST

The STLV E96A kit is based on the principle of indirect enzyme linked immunoassay. Microwells are coated with purified HTLV-1 antigen, which is a suitable alternative for STLV antibody detection in non-human primates sera. After adding testing samples into the wells, any STLV-1 antibody present in the specimens will specifically bind to the coated antigen. After removal of unbound material, anti-human IgG conjugated horseradish peroxidase is added to react with the immuno-complex. After removal of the extra conjugate by washing, a HRP substrate is added to have color development. Lastly, the test plate is read in a spectrophotometer to determine if STLV-1 antibody is present in sera.

## 9. TEST RESULTS AND INTERPRETATION

**9.1. Validation:** The test result is valid when the Negative Control is negative and the Positive Control is positive.

If these criteria are not met, the test is not valid and must be repeated.

**9.2. Cut-off Value:** COV = 0.3

**9.3. Positive:** O.D. of specimen  $\geq$  COV

**9.4. Negative:** O.D. of specimen  $<$  COV

Any specimen O.D. between 0.20 and 0.36 is suggested to be retested with another confirmatory procedure, e.g., STLV Western Blot (VRL Cat # W-080118-020) or PCR. Samples can be submitted to VRL Laboratories for confirmatory testing.

## 8. TEST PROCEDURE

Equilibrate all reagents to room temperature before use. Label each strip on its end tab to identify the strips should they become detached from the plate frame during the assay.

- 8.1. Sample Dilution (1:50)** Place testing serum or plasma and provided serum control tubes on a test tube rack. Pipet 490 µl of sample diluent (5% NFM) per well into a deep well sample dilution plate. Add 10 µl each of control sera and testing samples into its assigned well, then seal the plate with a plate sealer and mix well on a mixer. Note: Change tips between specimens to avoid cross contamination.
- 8.2. Specimen Incubation** Set up one negative control well and one positive control well in each individual test run. Add 100 µl of each diluted testing sample or controls into each well and then seal the plate with a plate sealer. Incubate at 37°C for half hour.
- 8.3. Washing** After incubation, remove 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.
- 8.4. Conjugate Incubation (1:1000)** Dilute provided secondary antibody conjugate 1:1000 in Wash Buffer by adding 12 µl of HRP Conjugate stock into 12 ml of wash buffer and mix well. Pipet 100 µl of diluted conjugate into each well. Cover the plate with the plate sealer and incubate at 37°C for half hour.
- 8.5. Washing** Aspirate and wash plate as described in step 8.3.
- 8.6. Color Development** Pipet 100 µl of HRP Substrate solution (read-to-use ABTS) into all wells. Incubate uncovered at room temperature (20-25°C) for half hour. A blue color will develop in wells containing STLV antibody.
- 8.7 Reading** Read the optical density (O.D.) of each well at 405 nm and use reference wave length at 490 nm to normalize well background.
- 8.8. Stop Reaction (optional)** If the plate is not read immediately, stop the reaction by pipetting 25 µl of Stop Solution into each well and agitate to mix. No color change will happen after stopping. Read the plate at 405/490 nm 2 min later but within 30 min.

## 3. MATERIALS

### 3.1. Materials supplied

Each STLV E96A kit provides sufficient reagents to run 96 tests:

**Antigen Coated Microplate:** 12x8 well strips on a strip frame in a foil pack for 96 tests.

**Concentrated Wash Buffer (20x):** 2 bottles (medium colorless), 25 ml each.

**Sample Diluent:** 5 g non-fat dry milk in a foil pack.

**HRP Conjugate:** 20µl goat anti-human IgG conjugated to horse-radish peroxidase (colorless top vial).

**Positive Control:** 50 µl STLV positive control sera (violet top vial).

**Negative Control:** 50 µl STLV negative control sera (green top vial).

**HRP Substrate:** 12 ml ready-to-use ABTS peroxidase substrate (amber bottle).

**Stop Solution:** 5 ml ready-to-use proprietary formulation (small colorless bottle).

NOTE: Store all reagents at 2-8°C.

### 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 (10, 100 and 1000µl) and multichannel (50-300 µl) and tips
- Validated incubator for 37°C
- Distilled water (dH<sub>2</sub>O) 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 U.V. Good laboratory practice, however, dictates that all materials be handled in accordance with practices employed in a bio-hazardous laboratory.
- Control sera provided 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 materials collected from animals 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 biohazard materials.
- Stop Solution contains 1.25% sodium fluoride. CAUTION: Avoid contact with eyes and skin. 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 2-8°C. At this temperature, shelf life is at least 6 months. An expiration date is provided with each kit when stored at 2-8°C.

Microplate strips in a frame are packed with desiccant in an aluminum bag.

If the entire 96 well plate is not used, remove strips from the strip frame. Place strips and desiccant in a press seal bag, close bag carefully to ensure airtight conditions and store at 2-8°C.

After opening, the strips are stable for 4 weeks at 2-8°C in a dry condition.

## 6. SPECIMEN PREPARATION

### 6.1. Collect Samples

Obtain blood and allow clot to form. Insoluble materials should be removed by centrifugation. Remove the serum aseptically.

Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be frozen. Avoid repeated freezing/thawing of samples.

Plasma samples (EDTA, citrate, heparin) can also be used. Samples should not contain sodium azide.

## 7. REAGENT PREPARATION

### 7.1. Prepare Wash Buffer (0.05%PBST, pH 7.2~7.4)

Check the wash concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilized by warming at 37°C until crystals dissolve.

Wash Buffer is stable for 3 weeks from the date of preparation if stored at 2-8°C. Therefore, dilute Concentrated Wash Buffer as needed.

Dilute the Concentrated Wash Buffer 1:20 with deionized or distilled water in a clean glass or plastic screw cap container (for example: add 50 ml Concentrated Wash Buffer into 950 ml of water) to make 0.05% PBST wash buffer. Mix gently by inverting several times to avoid excessive foaming.

### 7.2. Prepare sample diluent (5% dry milk in wash buffer)

Add 5 g of sample diluents (non-fat dry milk, NFM) into 100 ml of wash buffer to make 5% sample diluent solution.

Sample diluents solution is stable for less than 2 days, even stored at 2-8°C. Therefore, prepare sample diluents as needed. Each well requires about 0.6 ml sample diluent solution.

Keep powder milk sealed and dry to avoid deteriorated.