### PRODUCT INFORMATION

<table>
<thead>
<tr>
<th>Pkg#</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96T</td>
</tr>
</tbody>
</table>

**Intended use**
The HTLV-I/II p19 Antigen ELISA Kit is an enzyme linked immunoassay for the detection of Human T-Lymphotropic Virus Type I (HTLV-I) and Type II (HTLV-II) core antigen in test specimens. The assay is useful in monitoring the course of expression of HTLV-I and HTLV-II in cell cultures and to monitor the purification and biochemical behavior of HTLV-I and HTLV-II. The HTLV-I/II p19 Antigen ELISA Kit may augment and/or supplant reverse transcriptase (RT) measurements traditionally used to assess the presence of retroviruses. Such enzymatic measurements, however, are not HTLV-I or HTLV-II specific. In contrast, the HTLV-I/II p19 Antigen ELISA Kit is immunologically specific for HTLV-I and HTLV-II, uses no radioactive components and is more sensitive than RT.

**General Description**
The Human T-lymphotropic virus Type I (HTLV-1) is a human RNA retrovirus that is known to cause a type of cancer, referred to as adult T-cell leukemia and lymphoma, and a demyelinating disease called HTLV-I associated myelopathy/Tropical spastic paraparesis (HAM/TSP). HTLV-I is one of a group of closely related primate T lymphotropic viruses (PTLVs). Members of this family that infect humans are called Human T-lymphotropic viruses, and the ones that infect old-world primates are called Simian T-lymphotropic viruses. To date, four types of HTLVs (HTLV-I, HTLV-II, HTLV-III, and HTLV-IV) and four types of STLVs (STLV-I, STLV-II, STLV-III, and STLV-V) have been identified. The HTLVs are believed to originate from intraspecies transmission of STLVs. HTLV-I is an abbreviation for the human T-cell lymphotropic virus type 1, also called the Adult T-cell lymphoma virus type 1, a virus that has been seriously implicated in several kinds of diseases, including HTLV-I-associated myelopathy and Strongyloides stercoralis, and as a virus cancer link for leukemia (see adult T-cell leukemia/lymphoma). A virus closely related to HTLV-I, HTLV-II shares approximately 70% genomic homology (structural similarity) with HTLV-I.

**Principle Of The Test**
Microwells coated with high affinity polyclonal antibodies form the capture phase of the assay. These antibodies react strongly with the major gag gene products of HTLV-I and HTLV-II. Viral antigen in the test specimen is captured by the antibody during the sample incubation step. Captured antigen reacts with Detector Antibody which recognizes p19 core protein of HTLV-I and HTLV-II. Specifically bound Detector Antibody is detected with peroxidase conjugated IgG and color is developed with 3,3′,5,5′-tetramethylbenzidine (TMB) as substrate. Resultant absorbance values are proportional to the amount of viral core antigen present in the test specimens.

**Reagents And Materials Provided**
1. HTLV Antibody Coated Microplate (1 plate): 96 well microplate coated with polyclonal antibodies.
2. HTLV I/II Detector Antibody (0.5ml): Antibodies to HTLV-I and HTLV-II p19 core proteins. Contains added protein, Triton X-100 and 2-chloroacetamide.
3. HTLV-I Antigen Standard (0.5ml): Detergent-disrupted, heat-inactivated viral antigen at a concentration of 16 ng/ml p19. Contains added protein, Triton X-100 and sodium azide.
4. Lysing Buffer (5ml): Triton X-100 in PBS and 2-chloroacetamide.
5. Peroxidase Reagent (0.3ml): Peroxidase conjugated IgG. Contains added protein, Triton X-100 and 2-chloroacetamide.
6. Assay Diluent (100ml): Contains PBS, added protein, Triton X-100 and 2-chloroacetamide.
7. 10X Plate Wash Buffer (125ml): Contains PBS, Tween 20 and 2-chloroacetamide.
8. Substrate (0.5 ml): Tetramethylbenzidine (TMB) solution in dimethyl sulfoxide.
10. Stop Solution (12ml): Proprietary formulation
11. Resealable Plastic Bag: 1

**Materials Required But Not Supplied**
1. Test tubes and racks for preparing specimen and control dilutions
2. Validated adjustable micropipettes, single and multichannel
3. Distilled or deionized water
4. Validated incubator capable of maintaining 37° ± 1°C
5. Timer
6. Graduated cylinders and assorted beakers
7. Validated microplate reader
8. Automatic microplate washer or manual vacuum aspiration equipment
9. 1% sodium hypochlorite as disinfectant. May be prepared from household bleach
**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**

HTLV-I ANTIGEN STANDARD:

Prepare a series of six standards, in Assay Diluent, from the HTLV-I Antigen Standard. The dilution scheme in the Table below is recommended. Table 1.

<table>
<thead>
<tr>
<th>Standard Number</th>
<th>Concentration of p19</th>
<th>Volume of HTLV-I Antigen Standard</th>
<th>Volume of Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>800 pg/ml</td>
<td>50 µl</td>
<td>950 µl</td>
</tr>
<tr>
<td>2</td>
<td>400 pg/ml</td>
<td>500 µl of #1</td>
<td>500 µl</td>
</tr>
<tr>
<td>3</td>
<td>200 pg/ml</td>
<td>500 µl of #2</td>
<td>500 µl</td>
</tr>
<tr>
<td>4</td>
<td>100 pg/ml</td>
<td>500 µl of #3</td>
<td>500 µl</td>
</tr>
<tr>
<td>5</td>
<td>50 pg/ml</td>
<td>500 µl of #4</td>
<td>500 µl</td>
</tr>
<tr>
<td>6</td>
<td>25 pg/ml</td>
<td>500 µl of #5</td>
<td>500 µl</td>
</tr>
<tr>
<td>7</td>
<td>0 pg/ml</td>
<td>0 µl</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

Any diluted HTLV-I Antigen Standard remaining after the completion of the assay should be discarded. Do not save diluted reagent.

HTLV-I/II DETECTOR ANTIBODY, PEROXIDASE, AND SUBSTRATE working solutions: 1/100 final dilution. The dilution scheme in the Table below is recommended. Dilute HTLV-I/II Detector Antibody and Peroxidase Reagent in Assay Diluent. Dilute Substrate in Substrate Buffer. Do not save diluted reagents. Table 2.

<table>
<thead>
<tr>
<th>Number of strips</th>
<th>Volume of Reagent</th>
<th>Volume of Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30 µl</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>6</td>
<td>60 µl</td>
<td>6.0 ml</td>
</tr>
<tr>
<td>9</td>
<td>90 µl</td>
<td>9.0 ml</td>
</tr>
<tr>
<td>12</td>
<td>120 µl</td>
<td>12.0 ml</td>
</tr>
</tbody>
</table>

PLATE WASH BUFFER:

Dilute 1:10 in distilled or deionized water prior to use. Mix thoroughly. Prepared 1X Plate Wash Buffer may be stored at 2o-8°C for up to 1 week. Additional Wash Buffer may be ordered.
Assay Steps

Allow all reagents to reach room temperature before use. 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, and store at 2o-8oC.

Label each strip on its end tab to ensure identity should the strips become detached from the plate frame during the assay.

Step 1: Specimens to be tested (serum, culture fluids, chromatographic or ultracentrifugation fractions, etc.) must be treated with Lysing Buffer. Pipet 50 μl of Lysing Buffer into 450 μl of specimen. Mix well.

Step 2: Wash the microplate prior to the addition of samples. Fill each well with 300 μl of 1X Wash Buffer and aspirate. Perform 6 fill/aspirate cycles. After final wash cycle, thoroughly striking the inverted microplate on a pad of absorbent towels placed on the bench top. Continue striking until no droplets remain in the wells. Do not allow washed plates to completely dry prior to sample addition. Drying will adversely affect test results.

Step 3: Designate one well of the microplate, and leave empty. This well is used for background determination (substrate blank).

Step 4: Pipet 200 μl of standards #1-7 into duplicate wells.

Step 5: Pipet 200 μl of each specimen into separate duplicate wells.

Step 6: Cover the microplate with a sealer and incubate the plate for 2 hours at 37oC.

Step 7: Aspirate the contents of each well; wash plate as described in Step 2.

Step 8: Pipet 100 μl of HTLV-I Detector Antibody working solution to each well of the microplate except the substrate blank which is left blank. See Preparation of Reagents for dilution information. Cover and incubate for one hour at 37°C.

Step 9: Aspirate the contents of each well; wash plate as described in Step 2.

Step 10: Pipet 100 μl of the Peroxidase working solution into each well except the substrate blank which is left blank. See Preparation of Reagents for dilution information. Cover and incubate for 1 hour at 37°C.

Step 11: Aspirate the contents of each well; wash plate as described in Step 3.

Step 12: Pipet 100 μl of Substrate Solution into each well and incubate uncovered for thirty minutes at room temperature (18-25°C). A blue color will develop in wells containing viral antigen.

Step 13: Stop the reaction by pipetting 100 μl Stop Solution into each well. A color change from blue to yellow will result.

Step 14: Within fifteen minutes, read the optical density of each well at 450 nm using a microplate reader.

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:

? The mean optical density of the 0 pg/ml standard and the substrate blank must be less than 0.200.

? The mean optical density of the 200 pg/ml standard must be greater than or equal to 0.500.

Calculation

Using linear graph paper, plot mean optical densities for each standard used on the Y axis versus the corresponding concentration of HTLV-I p19 (pg/ml) on the X axis.

Determine the concentration of HTLV-I p19 in specimens by interpolation from the standard curve. Correct sample values for the 1.1 fold dilution made by the addition of Lysing Buffer and for any other dilutions performed during specimen preparation.

Precautions

1. Use Universal Precautions when handling test specimens and when performing this test.
2. When examining human source material or other potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding disposal of hazardous materials. To avoid cross-contamination, use separate pipet tips for each specimen.
3. The HTLV-I 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.
4. 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.
This is an example of a typical standard curve. Variation may occur in individual labs due to pipetting, laboratory and incubator temperatures, etc.

Table 3.

<table>
<thead>
<tr>
<th>HTLV-I p19 Antigen Concentration</th>
<th>Optical Density O.D. Mean at 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>800 pg/ml</td>
<td>1.746</td>
</tr>
<tr>
<td>400 pg/ml</td>
<td>1.167</td>
</tr>
<tr>
<td>200 pg/ml</td>
<td>0.698</td>
</tr>
<tr>
<td>100 pg/ml</td>
<td>0.394</td>
</tr>
<tr>
<td>50 pg/ml</td>
<td>0.207</td>
</tr>
<tr>
<td>25 pg/ml</td>
<td>0.108</td>
</tr>
<tr>
<td>0 pg/ml</td>
<td>0.050</td>
</tr>
</tbody>
</table>