The HIV 1/2/O Antigen/Antibody EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HIV-1 P24 antigen and total antibodies (IgG, IgM and IgA) to HIV-1, HIV-2, and Subtype O in human serum or plasma. The kit utilizes HIV mononuclear antibodies and recombinant antigens to selectively detect HIV-1 P24 antigen and antibodies to HIV-1, HIV-2 and Subtype O in serum or plasma.

**HEALTH AND SAFETY INFORMATION**

- For professional in vitro diagnostic use only.
- Do not use after expiration date.
- Avoid cross contamination between reagents to prevent false test results.
- Follow the wash procedure to prevent carry-over.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the wells.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingers.
- Do not use sodium hypochlorite hypochlorous from chlorine ble时尚or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

**INTENDED USE**

The HIV 1/2/O Antigen/Antibody EIA Test Kit is a qualitative enzyme immunoassay for the detection of HIV-1 P24 antigen and total antibodies (IgG, IgM and IgA) to Human Immunodeficiency Virus (HIV) type 1 and 2, and Subtype O in human serum or plasma. For professional in vitro diagnostic use only.

**SUMMARY**

- An enzyme immunoassay (EIA) for the qualitative detection of HIV-1 P24 antigen and total antibodies (IgG, IgM and IgA) to Human Immunodeficiency Virus (HIV) type 1 and 2, and Subtype O in human serum or plasma.
- It is intended for screening and as an aid in the diagnosis of possible HIV infection.

**PRINCIPLE**

The HIV 1/2/O Antigen/Antibody EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HIV-1 P24 antigen and total antibodies (IgG, IgM and IgA) to HIV-1, HIV-2, and Subtype O in human serum or plasma. The kit utilizes HIV mononuclear antibodies and recombinant antigens to selectively detect HIV-1 P24 antigen and antibodies to HIV-1, HIV-2 and Subtype O in serum or plasma.

**SPECIES COLLECTION AND PREPARATION**

- For professional in vitro diagnostic use only.
- Do not use after expiration date.
- Avoid cross contamination from other kits with different lot numbers.
- Avoid cross contamination between reagents to prevent false test results.
- Follow the wash procedure to prevent carry-over.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.

**REAGENTS AND COMPONENTS**

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Automated processor (optional)
- Note:
  - Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingers.
  - Do not use sodium hypochlorite hypochlorous from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
  - All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

**STORAGE AND STABILITY**

- Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through expiration date printed on the box stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the reagent(s) from the pouch. Store at 2-8°C until use.
- Note:
  - Do not store Stop Solution in a shallow dish or return it to the original bottle.
  - Leave A1 as Blank well.

**SPECIMENS**

- HIV-1/2/O Antigen
- HIV-1/2/O Antibody

**PROCEDURE**

- The HIV 1/2/O Antigen/Antibody EIA Test Kit is a solid phase enzyme immunoassay based on a sandwich principle for the detection of HIV-1 P24 antigen and total antibodies (IgG, IgM and IgA) to Human Immunodeficiency Virus (HIV) type 1 and 2, and Subtype O in human serum or plasma. The kit utilizes HIV mononuclear antibodies and recombinant antigens to selectively detect HIV-1 P24 antigen and antibodies to HIV-1, HIV-2 and Subtype O in serum or plasma. The enzyme conjugated HIV polyclonal antibodies are used in the assay. A reference filter is provided with the kit.

**TROUBLESHOOTING**

- Do not use the reagents beyond the expiration date.
- Do not store in the refrigerator of freezer after opening the pouch.
- Do not use any of the reagents beyond the expiration date.

**QUALITY CONTROL**

- HIV-1 Positive Control
- HIV-2 Positive Control
- Subtype O Positive Control

- HIV-1 Negative
- HIV-2 Negative
- Subtype O Negative

- HIV-1/2/O P24

**QUESTIONS FOR CLINICS**

- Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. The controls for each assay should be within the acceptable range for the configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
<th>Simplified Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:30</td>
<td>Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25</td>
</tr>
<tr>
<td>2</td>
<td>Prepare Working Enzyme Conjugate using contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill with 100 μL of Working Enzyme Conjugate for each 96 wells/plate testing. The Working Enzyme Conjugate is stable by itself for 2 weeks at 15-30°C.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Add 98 μL of Working Wash Buffer to plate for 96 wells, 49 μL of Working Wash Buffer to plate for 48 wells</td>
<td>Remove and store unused strips at 2-8°C</td>
</tr>
<tr>
<td>4</td>
<td>Remove unused strips from the microwell plate, and store in the original disposable pouch at 2-8°C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Discard all contaminated material, specimens and reagents of human origin after proper decontamination</td>
<td></td>
</tr>
</tbody>
</table>
1. The HIV 1/2/O Antigen/Antibody EIA Test Kit is designed for the detection of HIV 1 P24 antigens and antibodies to HIV-1, HIV-2, and/or Subtype O and may be considered negative.

2. Positive: 148 2 148

3. Negative: 0 1,240 1,240

4. The Positive Controls in the test kit are not to be used to quantify assay sensitivity. The Positive Controls are used to verify that the test components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HIV 1/2/O Antigen/Antibody EIA Test Kit has correctly identified specimens of a seroconversion panel and has been compared to a leading commercial HIV EIA test using clinical specimens. The results show that the clinical sensitivity of the HIV 1/2/O Antigen/Antibody EIA Test Kit is > 99.9%, and the clinical specificity is 99.9%.

LIMITATIONS

1. The HIV 1/2/O Antigen/Antibody EIA Test Kit should not be used for the detection of HIV 1 P24 antigens and antibodies to HIV-1, HIV-2, and/or Subtype O in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.

2. With all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

3. As with other sensitive immunossays, there is the possibility that non-repeatable reactive reactions may occur due to inadequate washing. The results may be affected due to procedural or instrument error.

4. The Positive Controls in the test kit are not to be used to quantify assay sensitivity. The Positive Controls are used to verify that the test components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control and Positive Controls by referring to the table below. Example of Negative Control Calculation

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Diluent</td>
<td>0.038</td>
</tr>
<tr>
<td>HIV-1 Positive Control</td>
<td>0.038</td>
</tr>
<tr>
<td>HIV-2 Positive Control</td>
<td>0.038</td>
</tr>
<tr>
<td>Substrate A</td>
<td>0.038</td>
</tr>
<tr>
<td>Substrate B</td>
<td>0.038</td>
</tr>
</tbody>
</table>

2. Check the validation requirements below to determine if the test results are valid.

**Method**

**HIV 1/2/O Antigen/Antibody EIA**

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0.038</td>
</tr>
<tr>
<td>Negative</td>
<td>0.038</td>
</tr>
</tbody>
</table>

**Other EIA**

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0.038</td>
</tr>
<tr>
<td>Negative</td>
<td>0.038</td>
</tr>
</tbody>
</table>

**Total Results**

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0.038</td>
</tr>
<tr>
<td>Negative</td>
<td>0.038</td>
</tr>
</tbody>
</table>

**Clinical Specificity:**

99.9% (99.5-100%) 95% Confidence Interval

**Intra-Assay**

**Within-run precision** has been determined by using 15 replicates of three specimens: a low positive, a medium positive, and a high positive.

**Inter-Assay**

Between-run precision has been determined by 3 independent assays on the same three