RESULTS
Calculate the mean absorbance for each control and unknown.

Qualitative results:
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG. Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:
- Positive: if the ratio is > 1.1.
- Doubtful: if +/- 10% of the Cut-Off.
- Negative: if the ratio is < 0.9.
If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE
- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL
Subtract the value of the blank from all the other readings. The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
104 human sera were analyzed by this HSV 1&2 IgG Elisa and a commercial Elisa (Test A) as reference method. Out of 104 samples, 83 were positive for the presence of IgG antibodies to Herpes simplex virus by DIAsource Elisa and commercial Elisa showed 83 of them as positive. The results are summarized below.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Positive</th>
<th>Negative</th>
<th>FN (false negative)</th>
<th>FP (false positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIA</td>
<td>83</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test A</td>
<td>83</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Precision

<table>
<thead>
<tr>
<th>Mean (OD’s)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates 16</td>
<td>Serum 1</td>
<td>0.315</td>
</tr>
<tr>
<td>Serum 2</td>
<td>1.92</td>
<td>0.074</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0.013</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean (OD’s)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates 16</td>
<td>Serum 1</td>
<td>0.261</td>
</tr>
<tr>
<td>Serum 2</td>
<td>0.55</td>
<td>0.015</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0.036</td>
<td>0.019</td>
</tr>
</tbody>
</table>

REFERENCE

CATALOG NUMBER: KAPRHGG24

PRODUCT INFORMATION
The DIAsource Herpes simplex virus 1&2 IgG ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

- HSV 1 & 2-Antigen-Coated Microtitration Strip
- Wash Concentrate
- Sample Diluent
- TMB-Substrate
- Negative control
- Cut off control
- Positive control
- 2nd Antibody Conjugate

REAGENTS

<table>
<thead>
<tr>
<th>Catalogue Nr.</th>
<th>PI Nr.</th>
<th>Revision Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAPRHGG24</td>
<td>1701199</td>
<td>110531/1</td>
</tr>
</tbody>
</table>
**MATERIAL NOT PROVIDED**

- Microtiter plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL, and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtiter plate washer
- Absorbent materials for blotting the strips
- Incubator capable of maintaining temperature at 37 +/- 2°C

**HSV 1&2 -Antigen-Coated Microtitration Strips**
One strip holder containing 12x8 (96) microtitration wells coated with Herpes simplex virus antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

**Wash Concentrate**
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

**Sample Diluent**
One bottle, 100 mL, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

**HSV 1&2 IgG Controls**
Three vials, negative, cut off and positive, each 2 mL of human serum with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

**2nd Antibody Conjugate**
One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

**TMB-Substrate**
One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

**Stopping Solution**
One bottle, 15 mL, containing 0.3 M H₂SO₄ in solution. Store at 2-8°C until expiration date.

**PRECAUTIONS**

For in vitro use

The following universal Good Laboratory Practices should be observed:

- Do not eat, drink or apply cosmetics where immunodiagnostic material is being handled. Do not pipe by mouth.
- Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards.
- Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces.
- Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

**WARNING: POTENTIAL BIOHAZARDOUS MATERIAL**

This kit may contain some reagents made with human source material (e.g., serum or plasma) or used in conjunction with human source materials. The kit in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential infectious human material in the Centers for Disease Control/ National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories,” 4th Edition, April 1999.

**PREPARATION FOR ASSAY**

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into all wells in the same order and speed to add the TMB Chromogen Solution. A void microbiological contamination of reagents, especially of the conjugate, wash buffer and diluent. A void contamination of the TMB Chromogen Solution with the Conjugate. Use a seal disposable pipette tip for each reagent. A void pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. A void exposure of the reagents to excessive heat or sunlight during storage and incubation.

**PREPARATION FOR REAGENTS**

**Wash Solution**

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

**Microtitration Strips**

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be ressealed to protect from moisture.

**ASSAY PROCEDURE**

All specimens and reagents to react room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtiter strips to be used.
2. Dilute serum samples 1:100 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
6. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
7. Add 100 µL of Enzyme-Labeled 2nd Antibody - Conjugate into each well.
8. Incubate for 45 minutes at 37°C.
9. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
10. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
11. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
12. Add 100 µL of Stopping Solution to each well using a dispenser.
13. Incubate for 30 minutes at room temperature (warm). A void exposure of the reagents to excessive heat or sunlight during storage and incubation.

**SPECIMEN COLLECTION AND HANDLING**

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

**WARNING**

Contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all hazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system. For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

**PREPARATION FOR ASSAY**

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into all wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbiological contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a seal disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

**PREPARATION FOR REAGENTS**

**Wash Solution**

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

**Microtitration Strips**

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be ressealed to protect from moisture.

**ASSAY PROCEDURE**

All specimens and reagents to react room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtiter strips to be used.
2. Dilute serum samples 1:100 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
6. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
7. Add 100 µL of Enzyme-Labeled 2nd Antibody - Conjugate into each well.
8. Incubate for 45 minutes at 37°C.
9. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
10. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
11. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
12. Add 100 µL of Stopping Solution to each well using a dispenser.

**WARNING**

Contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all hazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system. For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consult instructions for use</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>Contains sufficient for n tests</td>
</tr>
<tr>
<td>Use by</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>Batch code</td>
<td>Catalogue number</td>
</tr>
</tbody>
</table>

Revision date: 2011-05-31