IGM Antibodies to Herpes Simplex Virus Type 2 (HSV-2) ELISA Quantitation Kit

Manual

Catalog number: 40-052-115036

For the quantitative determination of IGM Antibodies to Herpes Simplex Virus Type 2 (HSV-2) in serum.

This kit is for research use only, and is not for use in diagnostic procedures.

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INTENDED USE

The GenWay Biotech HSV-2 IgM ELISA is intended for the detection of IgM antibodies to herpes simplex virus HSV-2.

INTRODUCTION

Herpes Simplex Virus is a common pathogen and its primary infection is usually asymptomatic. There are two immunologically distinct types of HSV: Type 1 and Type 2. HSV-1 is generally associated with oral infection and lesions above the waist, and HSV-2 is associated with genital infections and lesions below the waist. Clinical cases primarily are (1) eczema herpeticum with eczematous skin changes with numerous lesions, (2) gingivo-stomatitis, and (3) herpes sepsis, almost only found in premature newborn infants. The GenWay Biotech HSV-2 IgM ELISA is an accurate serologic method to detect HSV-2 IgM specific-antibody in serum samples.

PRINCIPLE OF THE TEST

Purified HSV-2 antigen is coated on the surface of microwells. Diluted serum is added to the wells, and the HSV-2 IgM-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:
- Antigen-Coated Wells (1 plate, 96 wells)
  Microtiter Wells coated with purified HSV-2 antigen
- Enzyme Conjugate Reagent (12 ml)
  Contains goat anti-human IgM conjugated to horseradish peroxidase with preservatives
- Sample Diluent (22 ml)
  Contains phosphate buffer with preservatives
- Negative Control (150 µl/vial)
  Contains HSV-2 IgM negative serum with preservatives
- Cut-off Calibrator (150 µl/vial)
  Contains HSV-2 IgM weak positive serum with preservatives; HSV-2 IgM Index = 1
- Positive Control (150 µl/vial)
  Contains HSV-2 IgM positive serum with preservatives
- Wash Buffer Concentrate (20x) (50 ml)
  Contains phosphate buffer and Tween 20
- TMB Reagent (11 ml)
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- Stop Solution -1N HCl (11 ml)
  Diluted hydrochloric acid

Materials required but not provided with the kit:
- Distilled or deionized water
- Precision pipettes: 5 µl, 100 µl, and 200 µl
- Disposable pipette tips
• Container: 1 liter
• Microliter well reader capable of reading absorbance at 450 nm
• Vortex mixer or equivalent
• Absorbent paper

**STORAGE CONDITIONS**

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

**WARNING AND PRECAUTIONS**

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984
2. This test kit is designed for research use.
3. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
4. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

**SPECIMEN COLLECTION AND PREPARATION**

1. The use of serum samples is required for this test.
2. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum samples.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).

**PROCEDURAL NOTES**

1. Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.
REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 μl of the sample to 200 μl of Sample Diluent. Mix well.
3. Dispense 100 μl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 μl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
6. Dispense 100 μl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
9. Dispense 100 μl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 μl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
   Note: Make sure there are no air bubbles in each well before reading.
13. Read O.D. at 450 nm **within 15 minutes** with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and experimental samples (x_s).
3. Calculate the HSV-2 IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c.

Example of typical results: **Note:** The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.
1. Calibrator O.D. = 1.010, 1.017  \( x_c = 1.014 \)

2. Negative Control O.D. = 0.048, 0.047  \( x_n = 0.048 \)
   HSV-2 IgM Index = \( x_n / x_c = 0.048/1.014 = 0.05 \)

3. Positive Control O.D. = 1.514, 1.667  \( x_p = 1.591 \)
   HSV-2 IgM Index = \( x_p / x_c = 1.591/1.014 = 1.57 \)

4. Experimental sample O.D. = 1.870, 1.871  \( x_s = 1.871 \)
   HSV-2 IgM Index = \( x_s / x_c = 1.871 / 1.014 = 1.85 \)

**QUALITY CONTROL**

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV-2 IgM Index for Negative and Positive Control should be in the range stated on the Certificate of Analysis.

**INTERPRETATION**

Negative: HSV-2 IgM Index less than 0.90 is negative for IgM antibody to HSV-2.

Equivocal: HSV-2 IgM Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: HSV-2 IgM Index of 1.00 or greater is positive for IgM antibody to HSV-2.

**PERFORMANCE CHARACTERISTICS**

I. Precision:

   A study was performed to document typical assay precision with the GenWay Biotech HSV-2 IgM EIA product. The mean, SD, and %CV were calculated for Intra- and Inter-Assay in O.D. value (A\text{450}).

   a. Intra-Assay Precision
   
   The following table presents the summary of the results of four (4) samples individually pipetted in groups of twenty-four (24) in a single assay.
b. Inter-Assay Precision
The following table presents the summary of the Inter-assay precision data determined by replicate testing of four (4) samples individually pipetted in groups of five (5).

<table>
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<tr>
<th>SERUM SAMPLE</th>
<th>MEAN (O.D. A&lt;sub&gt;450&lt;/sub&gt;)</th>
<th>S.D.</th>
<th>% C.V.</th>
<th>N</th>
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**LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

**REFERENCES**


Troubleshooting

The following are some common problems encountered with the use of ELISA kits, and some of the causes of these problems.

1. **Problem: Low absorbance**
   - Incorrect dilutions or pipetting errors.
   - Improper incubation times.
   - Improper mixing of the TMB substrate. Each component is mixed in equal parts.
   - Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
   - Kit materials or reagents are contaminated or expired.

2. **Problem: High Absorbance**
   - Cross contamination from other samples or positive control.
   - Incorrect dilutions or pipetting errors.
   - Improper washing.
   - Wrong filter on microtiter reader.
   - Contaminated buffers or enzyme substrate.
   - Improper incubation times.
   - Kit materials or reagents are contaminated or expired.

3. **Problem: Poor Duplicates**
   - Poor mixing of specimens.
   - Incorrect dilutions or pipetting errors.
   - Technical error.
   - Inconsistency in following ELISA protocol.
   - Inefficient washing.

4. **Problem: All wells are positive**
   - Contaminated buffers or enzyme substrate.
   - Incorrect dilutions or pipetting errors.
   - Kit materials or reagents are contaminated or expired.
   - Inefficient washing.

5. **Problem: All wells are negative**
   - Procedure not followed correctly.
   - Contaminated buffers or enzyme substrate.
   - Contaminated conjugate.
   - Kit materials or reagents are contaminated or expired.