# *ImmunoWELL* ™

## HSV 1+2 IgM TEST

CE

**Product No. 3600**

IVD **For *In Vitro* Diagnostic Use**

### INTENDED USE

ImmunoWELL HSV 1+2 IgM Test is an ELISA method for the qualitative detection of IgM antibody to Herpes Simplex Virus (HSV) in human serum. The results can serve as an aid in the diagnosis of HSV disease.

### SUMMARY AND EXPLANATION

HSV infections are caused by two antigenic types: type 1 or type 2. The serotype is determined using IgG antibodies. HSV infections are classified as either primary (first time) or recurrent. After the primary infection a latent infection is established and the latent infection may reactivate to cause recurrent infection.[1]

ImmunoWELL HSV IgG typing tests (Product codes: 3580 and 3590) are used to serologically determine past HSV exposure by type while ImmunoWELL HSV 1+2 IgM Test is used to detect specific IgM.

### ASSAY PRINCIPLE

The ImmunoWELL Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum from which IgG antibodies have been removed is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgM antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with tetramethylbenzidine (TMB), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a microwell spectrophotometer.

### REAGENTS

**Reaction Wells** coated with purified (glysine lysate) HSV types 1 & 2

**Specimen Diluent** - antihuman IgG in 0.01M phosphate buffered saline (PBS, pH 6.6-7.8), carrier protein and <0.1% $NaN_3$

**HSV IgM Calibrator** - human anti–HSV prediluted, ready for use in 0.01M PBS, carrier protein and <0.1% $NaN_3$

**HSV IgM Positive Control** - human anti–HSV serum containing <0.1% $NaN^3$

**HSV Negative Control** - nonreactive human serum containing <0.1% NaN3

**Wash Buffer Concentrate** consisting of a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween

**Conjugate** - peroxidase-conjugated goat antihuman IgM in PBS (pH 6.6-7.8) and carrier protein containing preservatives

**Substrate** - tetramethylbenzidine (TMB).

**Stop Solution** - 0.5 N Hydrochloric acid

#### Warnings and Precautions

**For In Vitro Diagnostic Use:** ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL Test reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Some reagents contain sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

**Warning - Potential Biohazardous Material:** Human sera used in the preparation of this product were tested using FDA approved procedures and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting disease.

#### Reconstitution and Storage

**Kit** is stored at 2-8°C until stated expiration date.

**Reaction wells** are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock. Unused wells must be used within six weeks of opening the pouch.

**Wash Buffer** (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.

> Note: In some instances the Wash Buffer Concentrate (20X) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely redissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

### SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown an acceptable specimen.

Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2–10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at –20°C.

### PROCEDURE

#### Materials Provided

| | |
|---|---|
| Microtiter Wells in Carrier | Specimen Diluent |
| Calibrator | Positive Control |
| Negative Control | Wash Buffer Concentrate (20X) |
| Conjugate | Substrate |
| Stop Solution | Package Insert |

#### Materials Required But Not Provided

| | |
|---|---|
| Distilled or deionized water | Pipets |
| Microwell washer | Test tubes |
| Microwell spectrophotometer (450 nm) | |

#### Performance Considerations

Reproducibility in the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.

**Positive and Negative Control Sera (Undiluted**) are used to assure test performance.

**Calibrator (prediluted**) is used to construct a standard curve.

**Substrate Blank** - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

#### Assay Procedure

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).
2. Determine the total number of specimens to be run. Include one blank and duplicates of calibrator and controls in each run.
3. For each control and specimen, pipet 10 µL serum into a clean tube containing 1 mL Specimen Diluent and mix (1:100 dilution).

**CAUTION: Calibrator is prediluted. Do not dilute further.**

4. Determine the total number of wells to be run including blank, calibrators, controls, and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.
5. Add 100 µL of Specimen Diluent into the first well as a substrate blank.
6. Pipet 100 µL of the prediluted calibrator and diluted controls and specimens (step 3) into each assigned well.
7. Incubate at room temperature (22–27°C) for 60±2 minutes.
8. Aspirate the samples out of the wells.

9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirating the wells completely after washes.
10. Pipet 100 µL Conjugate into all wells.
11. Incubate the wells at room temperature (22–27°C) for 30±2 minutes.
12. Aspirate the conjugate out of the wells.
13. Wash the wells three times as described in step 9.
14. Pipet 100 µL of Substrate into each well.
15. Incubate at room temperature (22–27°C) for 30±2 minutes.
16. Add 100 µL of Stop Solution to each well.
17. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution that might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.
18. Using the substrate blank to zero the spectrophotometer, read the optical density of each well at 450 nm within 30 minutes of completion of step 16.

### Quality Control

GenBio provides positive and negative controls with defined ranges provided in a Supplement included in the kit. The positive control value is approximately five standard deviations (absorbance) above the upper cutoff and the negative control value is less than 0.15 absorbance units. Interpretations should not be made unless the control results fall within these limits.

NCCLS C24-A should be consulted for guidance on appropriate quality control practices. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

## INTERPRETATION

### Procedure for Calculating Activity of Specimen

ImmunoWELL calibrator is assigned value of antibody concentration (U/mL) which may vary by lot number. Please verify that the lot number on the vials match the lot number on the Package Insert Supplement to assure the proper value is used in the calculation.

Calculate activity of the specimen by normalizing to the Calibrator according to the following:

$$V_S = A_S \times V_{MC}/A_{MC}$$

Where:

$V_S$ = Value of the specimen (U/mL)

$A_S$ = Absorbance of the specimen

$V_{MC}$ = Assigned Value of the Calibrator (U/mL) given in the Supplement

$A_{MC}$ = Mean absorbance of the Calibrator obtained in the assay

The interpretive ranges are:

| | Units/mL | Interpretation |
|---|---|---|
| Negative | <350 | Specific Antibody not detected |
| Equivocal | 350-400 | Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal. |
| Positive | >400 | Specific antibody detected |

The cutoff level is defined relative to the mean of negative sera. Clinical interpretation requires knowledge of the patient's condition. The higher the magnitude of a measured result above the cutoff, the more reliable positivity is assured.

Negative results do not rule out diagnosis. The specimen may have been drawn before appearance of detectable antibodies.

## LIMITATIONS

A diagnosis should not be made on the basis of anti-HSV IgM results alone.

## EXPECTED RESULTS

Prevalence may vary depending on geographical location, age, socioeconomic status, race, type of test employed, and other epidemiological and clinical criteria used to select patients.

## PERFORMANCE CHARACTERISTICS

**The relative sensitivity is 99% (96-99.5%) and the specificity is 95% (89-98%).**

Three hundred thirty-six (336) samples submitted to a reference laboratory during three months were evaluated. These samples were initially tested using the EIA kit (Kit A) used by the laboratory. Discrepant samples were evaluated using a second EIA kit (Kit B). Equivocal (borderline) samples were not used to calculate performance.

**Table 1: Relative Comparison to Commercial EIA**

| | | Reference Results | |
|---|---|---|---|
| | | Negative | Positive |
| *ImmunoWELL* | Negative | 97 | 3 |
| | Positive | 5 | 200 |

Note: Equivocal (borderline) samples were not used to calculate performance. Kit A reported 24 (7.14%) equivocal and GenBio reported 7 (0.02%) equivocal results.

### Precision Data

Precision was determined by testing seven samples as triplicates in three different runs. Values are expressed as coefficient of variation (percent).

**Table 2: Assay Precision**

| | Mean (units/mL) | Within Run | Between Run |
|---|---|---|---|
| A | 61 | 5.2% | 6.2% |
| B | 49 | 4.9% | 5.0% |
| C | 39 | 5.0% | 4.8% |
| D | 29 | 4.6% | 5.0% |
| E | 23 | 6.7% | 6.7% |
| F | 11 | 4.2% | 4.2% |
| G | 6 | 5.8% | 8.5% |

## BIBLIOGRAPHY

1. Arvin, Ann M., and Charles G. Prober. Chapter 71: Herpes Simplex Viruses in Manual of Clinical Microbiology ASM Press, 1995. 876-883.

**QUICK REFERENCE PROCEDURE**

***ImmunoWELL* HSV 1+2 IgM**

- Prepare Wash Buffer from Wash Concentrate.
- Dilute each control and specimen 1:100 in Specimen Diluent.
- Add 100 µL of Specimen Diluent into the first well as a substrate blank.
- Pipet 100 µL of the prediluted calibrator, and diluted controls and specimens into coated microwells and incubate 60 minutes at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipette 100 µL of Conjugate into microwells and incubate 30 minutes at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Substrate into microwells and incubate 30 minutes at room temperature.
- Pipet 100 µL Stop Solution into microwells and read results at 450 nm.

To place an order for ImmunoWELL products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.

For assistance, please call toll-free 800-288-4368.

GenBio

15222-A Avenue of Science

San Diego, CA 92128

EC REP

**EMERGO EUROPE**

Molenstraat 15

2513 BH, The Hague

The Netherlands