

Instruction Manual

Herpes 1/2 IgM ELISA

Enzyme immunoassay for the detection
of human IgM antibodies against **Herpes 1/2**
in serum and plasma



Cat. No.: IB79246
Storage: 2-8°C

RUO

USA: For research use only

Product information



Contents	Page
1. Intended Use	3
2. Summary and Explanation	3
3. Principle of the Test	3
4. Limitations, Precautions and General Comments	4
5. Reagents Provided	4
6. Materials Required but not Provided	5
7. Specimen Collection and Handling	6
8. Assay Procedure	6
9. Calculation of Results	7
10. Performance Characteristics	7
11. References	8

Product information



1. Intended Use

The IBL - AMERICA Herpes 1/2 IgM Antibody ELISA Test Kit has been designed for the detection of IgM antibodies against Herpes 1/2 in serum and plasma.

2. Summary and Explanation

The Herpes simplex virus type 1 is an ubiquitous pathogen of humans that usually causes either asymptomatic infection or mild skin and mucosal diseases. Antibodies to HSV 1 occur in about 90% of adults. Normally HSV 1 is transmitted by oral secretions or open wounds prior to the age of five. Recently in adults primary infections were observed, too. After the primary infection some viruses establish a latent state in their host cells (mostly ganglial cells). The virus DNA is integrated into the genome of the host cell, where it remains until the infected person dies. After stimulation of the host cell, recurrent infection occurs, which is called an exacerbation, when clinical symptoms appear. The recurrence may be caused by different kinds of traumas, as fever or physiological changes and diseases. Immunosuppressed persons may show a severe clinical course. HSV 1 causes different clinical symptoms in about 10% of the primary infections. HSV 1 causes 85% and HSV 2 15% of oral primary infections. The major clinical manifestations associated with HSV 1 infections are gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and some lethal infections of newborns. In some cases HSV 1 infection leads to a meningitis with different neurological symptoms. Persons at an increased risk for serious or prolonged HSV infections are those with eczema, severe burns or a defect in their cell-mediated immunity. The drug Acyclovir is the treatment of choice for most serious HSV infections. The common manifestations of HSV infections are so typical that the infection may be easily diagnosed on clinical recognition alone. The "gold standard" for diagnosis of HSV infection remains isolation of the virus in tissue culture. For typing HSV 1/HSV 2 Western blots or indirect immunofluorescence may be performed. Diagnosis of the primary infection by HSV 1/HSV 2 can be confirmed by a significant rise of the IgG titer within 6 to 10 days. A finished infection can be monitored by the IgG ELISA. In case of a suspicion of HSV encephalopathy it is recommended to perform a parallel determination of both HSV-specific antibodies (IgG and IgM) in serum and liquor.

3. Principle of the Test

The IBL - AMERICA Herpes 1/2 IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Herpes 1/2 antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Herpes 1/2 antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

Product information



4. Limitations, Precautions and General Comments

- Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.
- This assay is manufactured for IBL-America.

5. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Herpes 1/2 antigen coated microtiter strips	12 X 8 wells
Calibrator A (Negative Control)	1 X 2 mL
Calibrator B (Cut-Off Standard)	1 X 2 mL
Calibrator C (Weak Positive Control)	1 X 2 mL
Calibrator D (Positive Control)	1X 2 mL
Enzyme Conjugate	1 X 15 mL
Substrate	1 X 15 mL
Stop Solution	1 X 15 mL
Sample Diluent	1 X 60 mL
Washing Buffer (10×)	1 X 60 mL
Plastic foils	2 ea.
Plastic bag	1 ea.

5.1. Microtiter Strips

Product information



12 strips with 8 breakable wells each, coated with a Herpes 1/2 antigen (Mixture of herpes simplex 1, strain MacIntyre, herpes simplex 2, strain G, from African green monkey kidney). Ready-to-use.

5.2. Calibrator A (Negative Control)

2 mL, protein solution diluted with PBS, contains no IgM antibodies against Herpes 1/2. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. Calibrator B (Cut-Off Standard)

2 mL human serum diluted with PBS, contains a low concentration of IgM antibodies against Herpes 1/2. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.4. Calibrator C (Weak Positive Control)

2 mL, human serum diluted with PBS, contains a medium concentration of IgM antibodies against Herpes 1/2. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.5. Calibrator D (Positive Control)

2 mL, human serum diluted with PBS, contains a high concentration of IgM antibodies against Herpes 1/2. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate

15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.

5.7. Substrate

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution

15 mL, 1 N acidic solution. Ready-to-use.

5.9. Sample Diluent

2 x 60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Washing Buffer

2 x 60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.11. Plastic Foils

2 pieces to cover the microtiter strips during the incubation.

5.12. Plastic Bag

Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

Product information



7. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. Assay Procedure

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. After removing the third repetition of wash buffer, always remove residual moisture by inverting the microtiter plate and repeatedly tapping forcefully on a paper towel.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and repeat step 4 entirely.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Incubate without covering at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Calculation of Results

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

	OD Value	corrected OD	Mean OD Value
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Product information



Substrate Blank	0.022		
Negative Control (Std A)	0.088/0.093	0.066/0.071	0.069
Cut-Off Standard (Std B)	0.512/0.530	0.490/0.508	0.499
Weak Positive Control (Std C)	1.020/0.985	0.998/0.963	0.981
Positive Control (Std D)	1.980/2.027	1.958/2.005	1.982

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values**.

9.1. Qualitative

The calculated absorptions for the patient sera, as mentioned above, are compared with the value of the cut-off standard (Standard B). If the value of the sample is higher than the value of the cut-off standard, that sample should be interpreted as a positive result.

For a value below the cut-off standard (which is assigned a value of 10U/mL) the sample should be interpreted as a negative result. If a sample value is in the range from 8U/mL to 12U/mL, it is suggested that the sample be interpreted as equivocal and it is recommended that the test be repeated in duplicate using the same sample or a new sample from the same patient, taken after 2-4 weeks. Both samples should be measured in parallel in the same run.

The positive control (Standard D) must show at least double the OD of the cut-off standard (Standard B).

9.2. Quantitative

The ready-to-use standards and controls of the Herpes Simplex 1/2 antibody kit are defined and expressed in arbitrary units (U/mL). This results in a semi-quantitative evaluation. The lot-specific control ranges and the standard values are indicated on the QC data sheet provided with the kit.

For a semi-quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be determined in relation to the optical density. It is also possible to use automatic computer programs.

10. Performance Characteristics

Herpes 1/2 ELISA	IgM
Intra-Assay-Precision	4.2 %
Inter-Assay-Precision	7.9 %
Inter-Lot-Precision	5.2 – 11.1 %
Analytical Sensitivity	1.12 U/mL
Recovery	85 – 93 %
Linearity	64– 121 %
Cross-Reactivity	No cross-reactivity to Measles, Mumps and Varicella.
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Clinical Specificity	100 %
Clinical Sensitivity	100 %

11. References

Product information



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