BIOGENIX
INC. PVT. LTD.

Herpes Simplex Virus (HSV Ⅰ) IgG Antibody Enzyme Immunoassay Test Kit
（ELISA）

INTENDED USE

This kit is a qualitative detection of human serum/plasma of HSV Ⅰ IgG . The kit is suitable for clinical screening and diagnosis of HSV Ⅰ infection in serum/plasma.

SUMMARY AND EXPLANATION

Anti-human IgG monoclonal antibody is adsorpted in solid phase to the polystyrene reaction microplate. If there is HSV Ⅰ IgG antibody in test sample, it binds to anti-human IgG monoclonal antibody coated in microplate, and then binds to the enzyme labeled antigen and forms antigen-antibody-antiantibody complex on surface of the microplate, and display blue color in corresponding well via the action of substrate. Therefore, it can detect specifically the HSV 1 IgG in human serum/plasma.

PRINCIPLE OF THE TEST

This kit uses indirect ELISA principle to detect HSV Ⅰ IgG. Purified HSV Ⅰ antigen is pre-coated on the microplate, the HSV Ⅰ IgG in sample will combine with antigen first, then combine with enzyme-labeled anti-antibody to form antigen-antibody-antiantibody complex, and shows blue color in the microplate. This kit is used for the specific detection of HSV Ⅰ IgG antibody in human serum/plasma.

COMPONENTS

Materials provided with the kit:

	96T		48T	
Coated Microtiter Plate	1 bag	12*8	1 bag	12*4
Conjugate	1 vial	6.5 mL	1 vial	3.5 mL
Wash Buffer Concentrate (40*)	1 vial	20 mL	1 vial	10 mL
Sample Dilution Liquid	1 vial	11 mL	1 vial	6 ml
Substrate A	1 vial	7 mL	1 vial	3.5 mL
Substrate B	1 vial	7 mL	1 vial	3.5 mL
Stop Solution	1 vial	6 mL	1 vial	3 mL
Negative Control	1 vial	1 mL	1 vial	1 mL
Positive Control	1 vial	1 mL	1 vial	1 mL
Closure Plate Membrane	3 sheet		3 sheet	

Note: different batches of reagent kit, and different component can not be exchanged for use. Once open, stable for 3 months at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay.

Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid haemolysis of the RBC. Care should be taken to ensure that the serum/plasma specimens are clear and not contaminated by microorganisms.

2. HSV Ⅰ IgG ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
3. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

TEST PROCEDURE

1. All reagents should be allowed to reach room temperature for 15 minutes before use.
2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.
3. Add 100μL Sample Dilution Liquid in the corresponding hole (Do not add in the blank well, negative control wells and positive control well.) The sample should be corresponding to the number of micro plate, each plate should be provided with negative control 2 wells, positive control 1 well and blank control 1 well. (If detect with dual wavelength detection, setting no blank control well is allowed). **Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control to avoid cross contamination.**
4. Add 10μL sample in the corresponding hole, mix throughly by using the pipette, add 100μL negative control and positive control to negative control holes and positive control hole (Do not add in the blank well).
5. Shake gently to mix for 30 s. Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing the plate.
6. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.
7. Respectively adding Conjugate 50μL (Do not add in the blank well) Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing the plate.
8. Repeat the wash step for 5 times as in step 6.
9. Add Substrate A 50μL and Substrate B 1 drop (50μL) (Do not add in the blank well). Incubate at 37 ℃ for 10 minutes with the sealing plate membrane sealing the plate.
10. Add 50μL Stop Solution to each well (Do not add in the blank hole). Mix gently by shaking, read theabsorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Set no blank holes is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

INTERPRETATION OF RESULTS

Colorimetry: Read O.D at 450nm with a microplate reader.

Mean negative control O.D≤0.1and positive control O.D≥0.8, the test is valid, otherwise the test is invalid.

Cut off=Mean negative control A x2.1 (Calculated by 0.05 when Mean negative control

O.D. is ＜ 0.09, calculated by actual value when Mean negative control O.D. is ＞0.09)

Positive Results: Sample O.D ≥ Cut-off O.D.

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HSV Ⅰ IgG has probably been detected using HSV Ⅰ ELISA. All initially reactive specimens should be retested in duplicates using HSV Ⅰ ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HSV Ⅰ IgG with HSV Ⅰ ELISA.

Negative Results: Sample O.D < Cut-off O.D.

Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HSV Ⅰ IgG has been detected with HSV Ⅰ ELISA, therefore the patient is probably not infected with HSV Ⅰ and the blood unit do not contain HSV Ⅰ IgG.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS OF PROCEDURE

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and can not be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PERFORMANCE CHARATERISTICS

1.Negative Specificity: All results should be negative when detecting national negative quality control samples with ELISA kits of anti-HSV I IgG.

2. Positive Specificity: All results should be positive when detecting national positive quality control samples with ELISA kits of anti-HSV I IgG.

3.Limit of detection: At least three sixth result should be positive when detecting national anti-HSV I IgG national limit quality control samples with the ELISA kits on anti-HSV I IgG, the matrix fluid should result in negative.

4. Precision : Repeat the test for 10 times with the quality control material, the CV should not over 15%.

5. Inter-assay: Repeat the test for the same sample with 3 batches test kit for 10 times respectively, the CV should not over 20%.

6. Specificity Analysis: Add 120 IU/mL rheumatoid factor, 1400 μmol/L bilirubin, and 34 mmol/L triglyceride into the sample did not interfere with the test results. The sample contained 20 g/L hemoglobin cause the result in false positives. Hemolysis samples were not recommended; There is no cross reaction occurs with Hepatitis B, Dasanyang, Hepatitis A virus IgM antibody, human herpesvirus capsid antigen IgM antibody, rubella virus IgM antibody, Mycoplasma pneumoniae IgM antibody, varicella zoster virus IgM antibody, HSV I IgM antibody, antinuclear anitbody and anti-mitochondrial antibody.

7.HOOK Effect: There is no HOOK effect on testing for sample with high concentration anti-HSV I IgG.

ATTENTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.
4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.
5. Please put the unused plate back into the bag, and store at 2～8℃.
6. Operate strictly according to the instruction, control of reaction time and temperature strictly.
7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.
8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.
9. Negative results of reagent do not rule out the possibility of HSV Ⅰ infection. Positive results must be combined with clinical information for analysis.
10. All the reagents were treated by inactivation, but still should be regarded as potentially infectious. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

STORAGE & VALIDITY

1. Store at 2-8℃.DO NOT FREEZE.
2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

REFERENCES

Chinese Pharmacopoeia
China Biological Products Procedures

	Keep in Dark Place		Keep
	Dry Do Not Reuse		Temperature Limitation
	Consult Instruction for Use		In Vitro Diagnostic Medical Product
	Batch Code		Contains Sufficient for <n> Tests
	Manufacturer		Date of Manufacture
	This Side Up		Fragile

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