






Anti Sperm Antibody IgG GENLISA™ ELISA

REF : KBD801

Ver 1.0


IVD

Enzyme Immunoassay for the Qualitative Determination of Anti-sperm antibody IgG in human serum and plasma.

| | | | |
|---|-----------------------------|---|--------------------------------|
| IVD | For In-vitro Diagnostic Use | REF | Catalog Number |
|  | Store At | LOT | Batch Code |
|  | Manufactured By |  | Biological Risk |
|  | Expiry Date |  | Consult Operating Instructions |

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REF KBD801

 96 tests

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Introduction:

Antisperm antibodies (ASAs) are naturally formed against sperm-associated antigenic proteins following the exposure of these proteins to immune cells. ASAs are to be present in both males and females in considerable amounts. ASAs are found in higher concentrations (approximately 9–12%) among infertile patients.

Sperm cells entering the lower female reproductive tract are considered to be allogeneic antigens by the female body. Under these circumstances these allo-antigens can initiate allo-immunity responses in the female body. These allo-antigens expose the female immune system to pathologic conditions, resulting in a unique inflammatory or allergic reaction, which leads to the production of antisperm antibodies (ASAs).

For human males, risk factors associated with ASA formation include the blood–testis barrier breakdown, surgical trauma, various microbial infections, proctitis and orchitis, testicular cancer, varicocele.

Intended Use:

The Anti Sperm Antibody IgG GENLISA™ ELISA is intended for the qualitative determination of Anti-Sperm antibody IgG in human serum and plasma.

Principle:

Anti Sperm Antibody IgG GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay which is designed to qualitatively detect Anti Sperm IgG antibody present in the human serum and plasma. Purified ASA antigen is pre-coated onto microwells. Samples and Controls are pipetted into microwells and Anti Sperm IgG antibody present in test sample binds to the antigen coated on the wells. And then enzyme labelled antibody conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution (A and B) is added to microwells and color develops proportionally to the amount of Anti Sperm IgG antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Positive Control - 1 ml
3. Negative Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (40X) Wash Buffer - 20 ml
6. Sample Diluent - 11 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5 ml of Wash Buffer (40X) to 97.5 ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Sample Diluent** to the respective sample wells.
3. Add **5 ul Sample** to the respective wells. Mix gently.
4. Dispense **50 ul Positive Control** and **50 ul Negative Control** to the control wells respectively.
5. Shake gently for 30 seconds to mix well. Incubate at 37°C for 20 minutes.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **50 ul of Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37°C for 20 minutes.
9. Repeat the Aspirate / Wash Step.
10. Add **100 ul of TMB Substrate** to all wells.
11. Incubate at 37°C for 10 minutes.
12. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off value (CO) = OD_{mean} of Negative Control x 3

Note: incase the OD_{mean} of Negative Control is < 0.100 then assume the same as 0.100

Positive Results: OD value \geq CO

Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that anti-sperm antibody has probably been detected using the ELISA. All initially reactive specimens should be retested in duplicates using the Anti-Sperm Antibody ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for anti-sperm antibodies with the Anti-Sperm antibody ELISA.

Negative Results: OD value $<$ CO

Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Anti-Sperm Antibody has been detected with the Anti-Sperm Antibody ELISA.

| Cut Off Value | OD _{mean} of Negative Control x 3 |
|---------------|--|
| Positive | \geq CO |
| Negative | $<$ CO |

Criteria of Validation:

Anti-Sperm antibody results are considered to be valid, if
OD of Positive Control $>$ Cut-Off Value

OD = Optical Density / Absorbance at 450nm

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:**Sensitivity:**

Limit of Detection: When detecting anti-sperm antibody limits, laboratory quality control positive samples diluted till 1:8 with the Anti-sperm Antibody ELISA kit should be in the positive.

Specificity:

The recombinant antigen used in the kit is specific for Sperm antibody.

Precision:

Intra-Assay: CV% \leq 15%.

Inter-Assay: CV% \leq 20%

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide ($<$ 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.



- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



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Regulatory Status:

| | |
|------------------|--------|
| CE Marked | Europe |
| FDA registered | USA |
| CDSCO registered | India |

SCHEMATIC ASSAY PROCEDURE

| | |
|----|---|
| 1 | All reagents should be allowed to reach room temperature before use. |
| 2 | Add 100 ul Sample Diluent to the sample wells . |
| 3 | Add 5 ul Sample to the respective sample wells . Mix gently. |
| 4 | Dispense 50 ul Negative Control and 50 ul Positive Control to the negative and positive wells respectively. |
| 5 | Shake gently for 30 seconds to mix well. Incubate at 37°C for 20 minutes . |
| 6 | Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly. |
| 7 | Add 50 ul of Enzyme Conjugate to each well except the blank well. |
| 8 | Seal the plate and incubate at 37°C for 20 minutes . |
| 9 | Repeat the Aspirate / Wash Step. |
| 10 | Add 100 ul of TMB Substrate to all wells. |
| 11 | Incubate at 37°C for 10 minutes . |
| 12 | Add 100 ul of Stop Solution . Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction. |

SYMBOLS KEY

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|---|-------------------------------|
|  | Microtiter Plate (8x12 wells) |
|  | Controls |
|  | Enzyme Conjugate |
|  | TMB Substrate |
|  | Stop Solution |
|  | Consult Instructions for Use |
|  | Catalog Number |
|  | Expiration Date |
|  | Storage Temperature |