IMMUNOPORT

SKU IP406705

ELISA KIT FOR HUMAN ANTI-SPERM ANTIBODY

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.3

INTENDED USE

The kit is an enzyme immunoassay for in vitro quantitative measurement of Anti-Sperm Antibody (AsAb) in human serum, plasma and other biological fluids.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standard [2 pcs of 200ng/mL each]
Standard Diluent [20mL]
Detection Reagent A [120µL each]
Assay Diluent A [12mL each]
TMB Substrate [9mL each]
30X Wash solution [20mL]
Stop Solution [6mL]
Plate Sealing Membrane [4pcs]
User manual [1pc]
Zipper bag [1pc]

METERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37 °C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

SUMMARY

The microtiter plate provided in this kit has been pre-coated with AsAb. Standards or samples are then added to the appropriate microtiter plate wells. After which Horseradish Peroxidase (HRP)-conjugated secondary antibody is added to each well. After TMB substrate solution addition, those wells that contain AsAb will exhibit a change in color and the others will show no color. The enzyme substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of AsAb in the samples is then determined by comparing the O.D. of the samples to the standard curve.

CHARACTERISTICS

Sample Type: Serum, plasma, and other biological fluids.

Detection Range: 3.12-200ng/mL
Minimum Detection Dose: 1.17ng/mL
Intra-Assay Precision: CV<9%
Inter Assay Precision: CV<11%

Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacture
Storage: 2-8°C
Once opened unused wells should be put into the sealed bag with a desiccant pack and may be sorted for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

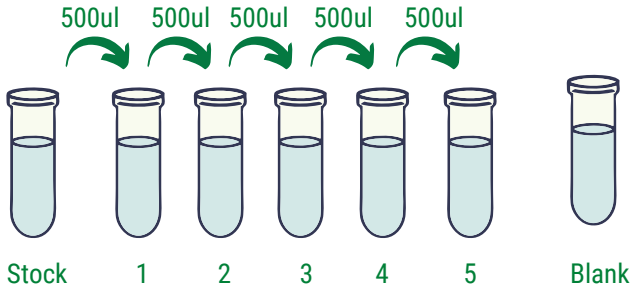
Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.
4. Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. **Standard** - Reconstitute the Standard with 0.9mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 200ng/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, and the last EP tube with Standard Diluent is the blank as 0ng/mL.



3. **Detection Reagent A** - Briefly spin or centrifuge the stock Detection A before use. Dilute to the working concentration with working Assay Diluent A, respectively (1:100).

4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C .

2. Remove the liquid of each well, don't wash.

3. Add 100μL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37°C after covering it with the Plate sealer.

4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 5 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.

6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with AsAb concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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