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IMMUNOPORT

IB05082

ELISA KIT FOR GOAT LEPTOSPIRA IGG

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

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VERSION 1.3

INTENDED USE

The kit is double antibody sandwich enzyme immunoassay for in vitro quantitative measurement of Leptospira Antibody IgG (Lep Ab IgG) in Goat serum, plasma, saliva, urine, cell culture supernates and other biological fluids.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standards A to F [0, 6.25, 12.5, 25, 50, 100 µg/ml]
Sample Diluent [6ml]
HRP-Conjugate reagent [10ml]
20X Wash solution [25ml]
Chromogen Solution A and B [6ml each]
Stop Solution [6ml]
Plate Sealing Membrane [2pcs]
User manual [1pc]
Sealedbag [1pc]

MATERIALS REQUIRED BUT NOT INCLUDED

Distilled Water, Pipettes and Tips, ELISA Reader, Eppendorf tubes, Absorbent paper and 37°C Incubator.

SUMMARY

This kit is based on the principle of double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). In brief, samples (and standards) containing the analyte are added to the micro-titre wells that are pre-coated with a protein which captures the analyte. Subsequently a HRP-conjugated reagent is added to form an immune complex. This is followed by incubation and washing, removal of unbound enzyme, and sequential addition of chromogen A and B which turns blue and finally changes into yellow under the effect of acid. The color density is positively correlated with the concentration of analyte.

CHARACTERISTICS

Sample Type: Serum, plasma, saliva, urine, tissue homogenate, cell culture supernates and other biological fluids.

Detection Range: 5µg/mL - 100µg/ml
Minimum Detection Dose: 0.5µg/ml
Intra-Assay Precision: CV<7%
Inter Assay Precision: CV<10%
Linearity with spiked analytes: ρ=0.99
Cross Reactivity: None observed with analogues
Validity: Mentioned on the box
Storage: 2-8°C

Once opened unused wells should be put into the sealed bag with a desiccant pack and stored at 2-8°C.

SAMPLE COLLECTION

Serum - Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully.
If sediments occur during storage, centrifugation should be performed again.

Plasma - In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulant. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments occur during storage, centrifugation should be performed again.

Urine - Collect urine in sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully.

Cell Lysates - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Pleuroperitoneal fluid and Cerebrospinal fluid - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

Cell Culture Supernatant - 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 24 hours 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.

PROCEDURE

1.Bring all reagents to room temperature before use. It is recommended that all Standards and Samples be performed in duplicate.

2.Set Standard wells and add standard 50µl to the standard wells. Set Sample wells and add 10µl samples to the sample wells, then add 40µl sample diluent to the sample wells. In Blank wells do not add anything.

3. Add 100µl of HRP-conjugate reagent to each well (Standard wells and Sample wells; but not in blank well), then cover it with seal plate membrane, gently shake and mix for 60 minutes at 37°C incubation.

5.Preparation of washing solution: Dilute the washing concentration (20X) with distilled or deionized water.

6. Wash the plate 5 times. To do manual washing, carefully remove the sealing film, drain the liquid by patting on absorbent paper. The fill each well with washing solution, hold for 1 min and then drain the liquid and pat on absorbent paper. To do automatic washing, inject each well with 350µL wash solution, soak 1 min, wash plate 5 times.

7. Add 50µl chromogen solution A to each well, then add 50µl chromogen B to each well. Shake gently to mix up. Incubate for 15 minutes at 37°C, away from light.

8. Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution. Calculate the concentration of the corresponding sample.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit.

As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the analyte concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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.

- Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

- It is highly recommended to use the remaining reagents within 1 month before the deadline. For the expiration date, please refer to the label on the kit box. All components are stable before this expiration date. Do not use kit components beyond their expiration date.

- 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.

- Substrate Solution is easily contaminated. If bluish prior to use, do not use. Substrate B is sensitive to light and avoid prolonged exposure to light.

- Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

- 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.

- Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

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