Version 17-05/24

User's Manual

Rat Leptospira IgG ELISA Kit

REF **DEIA05738**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

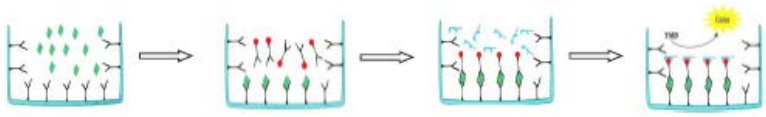
PRODUCT INFORMATION

Intended Use

The Rat Leptospira IgG (Lep IgG) ELISA Kit is to be used for the vitro quantitative determination of Rat Lep IgG in Serum, Plasma and other biological fluids. The Kit is intended for research use only, not for diagnostic or therapeutic procedure. If detection of other special sample, please contact our technical support.

General Description

Leptospires are spiral-shaped, Gram-negative, obligate aerobic spirochetes with internal flagella. The genus is divided into two species, the pathogenic Leptospira interrogans and the free-living nonpathogenic Leptospira biflexa. Leptospira interrogans has about 200 different serovars based on the variability of surface antigens. Leptospires affect mammals (wild and domestic animals), reptiles and amphibians; they may be shed in the urine lifelong. Rats and other rodents are primary reservoirs for human infection. Infection is transmitted by urine-contaminated soil or water, rat bites or animal tissue. Especially occupational groups like agriculturists, plumbers, mine workers, fishermen and meatindustry workers are at great risk of exposure.



Principles of Testing

The kit assay Rat Lep IgG level in the Samples, use Purified Rat Lep IgG antigen to coat microtiter plate wells, make solid-phase antigen, then add Samples (Containing Rat Lep IgG) to wells, combined Rat Lep IgG antigen which with HRP labeled, become antigen - antibody - enzyme-antigen complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change (yellow) is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Rat Lep IgG in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

1. Microelisa strip plate: 12 well×8 strips
2. Wash solution: 20 mL×1bottle(30 fold)
3. HRP-Conjugate reagent: 6 mL×1 bottle
4. Sample diluent: 6 mL×1 bottle
5. Chromogen Solution A: 6 mL×1 bottle
6. Chromogen Solution B: 6 mL×1 bottle
7. Standard: 2700 pg/ml, 0.5 mL×1 bottle
8. Standard diluent: 1.5 mL×1 bottle
9. Stop Solution: 6 mL×1bottle

10. Instruction: 1 pcs
11. Closure plate membrane: 2 pcs
12. Sealed bags: 1 pcs

Materials Required But Not Supplied

1. Microplate reader (450 nm detection wavelength filter, 570 nm or 630 nm correction wavelength filters)
2. Beakers, flasks, cylinders necessary for preparation of reagents
3. Clean benches, Incubator(37°C), Refrigerators (4°C, -20°C), Low Temperature Centrifuge
4. High-precision single-channel and multi-channel Pipette and disposable Tips.
5. Polypropylene tubes for diluting and aliquoting Standards if needed
6. Distilled water or de-ionized water7.Absorbent paper for blotting the microtiter plate8.Automated or manual microplate washer

Storage

All the components in the kit should be stored up to 6 months at 2-8°C, and should be kept according to the labels on vials.

Specimen Collection And Preparation

Serum: Allow samples to clot in a serum separator tube for two hours at room temperature or overnight at 4°C. Centrifuge at approximately 2000 -3000rpm for 15-20 min. Analyze the serum immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge at approximately 2000-3000 rpm for 15-20 min. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Tissue homogenates: After cutting samples, check the weight, and add PBS (PH7.2-7.4), rapidly freeze with Liquid Nitrogen; maintain samples at 2-8°C after melting, add PBS (PH7.4), homogenized in ice water. Centrifuge at approximately 2000-5000 rpm for 20 min. Having the Supernatant and discarded following precipitation. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatant: If detect the secretory components, please collect with a sterile container, then centrifuge at approximately 2000-3000 rpm for 20 min ,having the Supernatant; if detect the Intracellular components, please dilute the Cell suspension with PBS (PH7.2-7.4), and the concentration reached 1000000 / ml, repeated freeze-thaw cycles or other method, let the cells release the Intracellular components, centrifuge at approximately 2000-3000 rpm for 20min, having the Supernatant. If precipitation appeared, centrifugal again. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Urine, Saliva, GCF, Cerebrospinal fluid and other body fluids: Collect with a sterile container,remove precipitation by centrifugation at approximately 2000-3000 rpm for 20 min. Analyze immediately or aliquot and store frozen at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note:

1. Other biological samples might be suitable for use in the assay, please inquire our Tech Support.
2. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowlyand mixed gently.
3. Samples to be used within 24 hours may be stored at 4°C, otherwise samples must be stored at -20°C (≤3month) or -80°C (≤6 months) to avoid loss of bioactivity and contamination.
4. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed orlipemic specimens.
5. The collection and extraction of Sample,recommend the customer to read the relevant literiture.
6. Can not detect the sample containing NaN3, because NaN3 inhibits the activity of HRP.
7. Please strictly follow the instructions and use our recommended dilution factor.

Assay Procedure

1. Preparation of the Standard: Set 10 Standard wells on the ELISA plates coated, label ① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ . Add Standard 100μL to ① ② , then add Standard diluent 50μL to ①②, mix ; take out 100μL from①②,and then add it to ③ ④ separately, then add Standard diluent 50μL to ③ ④ ,mix ; then take out 50μL from ③ ④ ,and discard, then take out 50μL from ③ ④ and add to ⑤ ⑥ , then add Standard diluent 50μL to the⑤⑥, mix ; take out 50μL from ⑤⑥ and add to⑦⑧, then add Standard diluent 50μL to the ⑦⑧ ,mix ; take out 50μL from the ⑦⑧ and add to ⑨⑩, add Standard diluent 50μL to⑨⑩, mix , take out 50μL from⑨⑩discard. After dilution, the total volume in each well is 50 μL, and the concentration is 1800 pg/mL, 1200 pg/mL, 600 pg/mL, 300 pg/mL, 150 pg/mL respectively.
2. Set wells separately: Set blank well (Please do not add Sample and HRP-Conjugate reagent to the blank comparison wells,other each step operation is same), and Testing sample well.
3. Add Sample: Add Sample Diluent 40μL to Testing sample well, then add testing sample 10μL (Sample final dilution is 5-fold). Please add Sample to the bottom of pre-coated well , do not touch the well wall as far as possible, and mix gently.
4. Incubate: After closing the plate with Closure plate membrane, incubate for 30 min at 37°C.
5. Prepare the Washing Buffer: 30-fold Wash Solution, diluted 30-fold with Distilled water until 600 mL, and reserve.
6. Washing:Uncover Closure plate membrane, discard Liquid, dry by swing, add Washing Buffer to each well, still for 30s then drain, repeat 5 times, dry by pat.
7. Add enzyme:Add HRP-Conjugate Reagent 50μL to each well, except the Blank well.
8. Incubate:Operation with 4.
9. Washing:Operation with 6.
10. Color:Add TMB Chromogen Solution A 50uL and then add TMB Chromogen Solution B 50uL to each well, mix gently, evade the light preservation for 15 min at 37°C.
11. Stop the Reaction:Add Stop Solution 50μL to each well, to stop the reaction(the blue color change to yellow color immediately).
12. Assay:Set the OD of Blank well as zero , read absorbance at 450nm after adding Stop Solution within

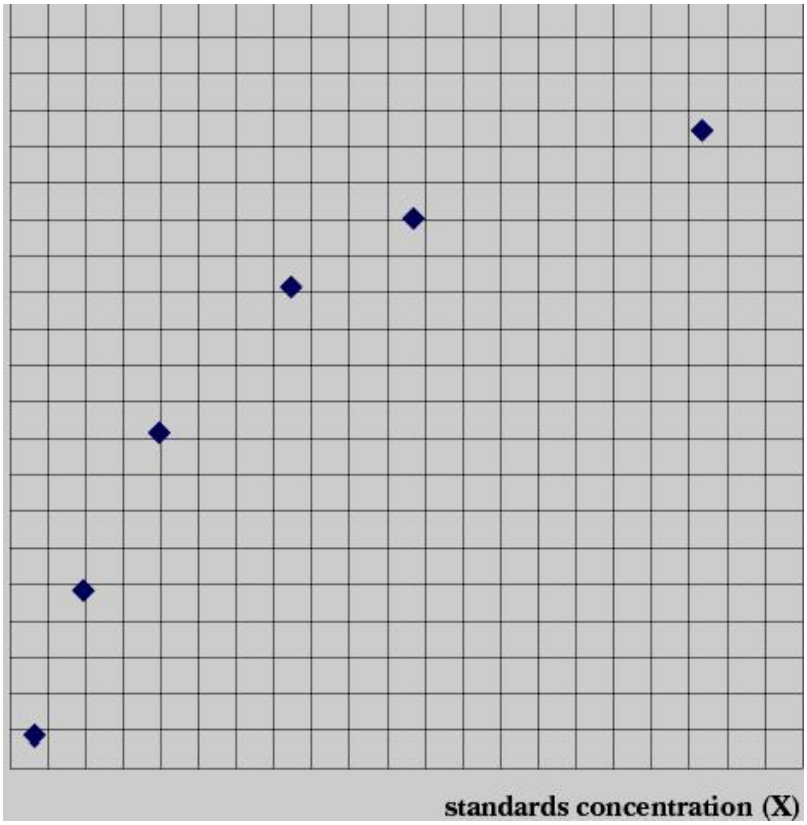
15min.

Note: The judgment of result must take the OD of Microplate reader as a standard, when use the dual-wavelength to assay, reference wavelength is 630nm.

Calculation

Take the Standard density as the horizontal, the OD value for the vertical, obtain the Standard Curve, then find out the corresponding density according to the sample OD value, and multiplied by the dilution multiple. Or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor,the result is the sample actual density.

Note: This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Detection Range

23.5 pg/mL- 2000 pg/Ml

Specificity

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. No significant cross-reactivity or interference between Rat Lep IgG and any of other cytokines.

Linearity

Correlation coefficient (R) of linear regression of the samples is more than 0.92 Hints.

Recovery

Intra-assay Precision (Precision within an assay): CV (%) = SD/mean X 100 ≤ 9%.

Inter-assay Precision (Precision between assays): CV (%) = SD/mean X 100≤15%.

Precautions

1. All the components in the kit should be stored up to 6 months at 2-8°C, and should be kept according to the labels on vials. (Storage:2-8 °C Validity: 6months)
2. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature then use.
3. The kit contain sufficient materials to run ELISAs on 96 or 48 microplates. Specific vial volume of each component may vary. Please check carefully if all components is in the correct volume.
4. Washing Buffer will present Crystallization separation, and it can be heated in the water helping to dissolve, which does not affect the result.
5. Closure plate membrane only limits the disposable use, in order to avoid the overlapping pollution.
6. Add sample within 5 min.
7. Please keeping the TMB Substrate evade the light preservation.
8. Upon receipt, foil pouch around the plate should be vacuum-sealed.
9. Please avoid repeated freeze-thaw cycles and do not mix reagents from different kits unless they have the same lot numbers.
10. After use remaining reagents should be returned to cold storage (2° to 8°C) immediately. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.
11. Do not use components beyond the expiration date. Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling. Expiry of the kit and reagents is stated on kit labels.
12. It is highly recommended to use the remaining reagents within 1 month provided, this is within the expiration date of the kit.
13. Any irregularities to aforementioned conditions may influence plate performance in the assay.