Version 06-11/24

User's Manual

Equine Relaxin-2 (RLN2) ELISA Kit

REF **DEIA-JY24091**

Σ **96T**

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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

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PRODUCT INFORMATION

Intended Use

This kit was used to determine the content of relaxin 2 (RLN 2) in horse serum, plasma, and associated liquid samples.

Principles of Testing

This kit employs a double antibody sandwich assay to quantitatively measure the levels of relaxin 2 (RLN2) in biological specimens. Purified RLN2 antibodies are immobilized on microtiter plates, establishing a solid-phase antibody layer. Following this, the sample containing RLN2 is introduced into the coated wells. Subsequently, HRP-labeled RLN2 antibodies are added, resulting in the formation of a stable antibody-antigen-enzyme-labeled antibody complex.

After extensive washing to eliminate unbound components, the substrate TMB is introduced, facilitating color development. The TMB substrate is catalyzed by HRP, initially producing a blue solution, which then transitions to yellow upon exposure to acidic conditions. The intensity of the resulting colorimetric change is directly proportional to the concentration of RLN2 present in the sample.

The absorbance (optical density, OD) is measured at a wavelength of 450 nm utilizing an ELISA reader. The concentration of relaxin 2 (RLN2) in the sample is subsequently calculated by referencing a standard curve generated from known RLN2 concentrations.

Reagents And Materials Provided

1. 30× Concentrated wash solution: 20 ml, 1 bottle
2. Enzyme Conjugate: 6 ml, 1 bottle
3. Antibody coated plate: 12 well, 8 strip
4. Sample dilutions: 6 ml, 1 bottle
5. Chromogenic substrate A: 6 ml, 1 bottle
6. Chromogenic substrate B: 6 ml, 1 / bottle
7. Stop solution: 6 ml, 1 bottle
8. Standard stock (1200 pg/ml): 0.5 ml, 1 bottle
9. Standard dilution: 1.5 ml, 1 bottle
10. Instructions: 1
11. Plate film: 2 sheet
12. Sealed bag: 1

Materials Required But Not Supplied

1. Pipettes and pipette tips.
2. Distilled water or deionized water.

3. Volumetric containers and pipettes for reagent preparation.
4. Paper towels or absorbent paper.
5. Multi-channel micropipettes or automated microplate washer.
6. Microplate shaker capable of 600 rpm.
7. Microplate reader capable of reading absorbance at 450 nm.

Storage

Up to 6 months when stored as recommended at at 2-8 °C.

Specimen Collection And Preparation

1. Extract the specimens as soon as possible after collection, following the relevant literature for extraction procedures. Experiments should be conducted promptly after extraction. If immediate testing is not feasible, specimens can be stored at -20°C, but repeated freeze-thaw cycles should be avoided.
2. Samples containing NaN3 cannot be tested, as NaN_3 inhibits the activity of horseradish peroxidase (HRP).

Reagent Preparation

Dilution of standard: This kit provides one original standard, which the user can dilute in a small tube according to the following chart:

Conc.	STD	
600 pg/ml	Standard #5	Add 150 µl of standard stock to 150 µl of standard dilution
300 pg/ml	Standard #4	Add 150 µl of Standard #5 to 150 µl of the standard dilution
150 pg/ml	Standard #3	Add 150 µl of Standard #4 to 150 µl of the standard dilution
75 pg/ml	Standard #2	Add 150 µl of Standard #3 to 150 µl of the standard dilution
37.5 pg/ml	Standard #1	Add 150 µl of Standard #2 to 150 µl of the standard dilution

Assay Procedure

1. **Sample Addition:** Set up blank wells (control wells without samples or enzyme-labeled reagents, with subsequent steps performed similarly), standard wells, and test sample wells. Accurately add 50 µl of standard solution to the standard wells on the enzyme-coated plate. For test sample wells, first add 40 µl of sample dilution, followed by the addition of 10 µl of the test sample (resulting in a final dilution of 5 times). Add the sample at the bottom of the wells, avoiding contact with the well walls, and gently mix by shaking.
2. **Incubation:** Seal the plate with a sealing film and incubate at 37°C for 30 minutes.
3. **Preparation of Wash Solution:** Dilute the 30x concentrated wash solution 30-fold with distilled water and set aside for use.
4. **Washing:** Carefully remove the sealing film and discard the liquid. Invert the plate to dry it, then fill each well with wash solution. Let it sit for 30 seconds before discarding the liquid. Repeat this process 5 times and then tap dry.

5. **Enzyme Addition:** Add 50 µl of enzyme-labeled reagent to each well, except for the blank well.
6. **Incubation:** Follow the same procedure as in step 2.
7. **Washing:** Follow the same procedure as in step 4.
8. **Color Development:** First, add 50 µl of Color Development Reagent A to each well, followed by 50 µl of Color Development Reagent B. Gently mix and incubate in the dark at 37°C for 10 minutes.
9. **Termination:** Add 50 µl of Stop Solution to each well to terminate the reaction (the color will change from blue to yellow at this point).
10. **Measurement:** Use the blank well as a zero reference and measure the absorbance (OD value) of each well at a wavelength of 450 nm. Measurements should be performed within 15 minutes after adding the stop solution.

Calculation

Plot the standard concentrations on the x-axis and the OD values on the y-axis to create a standard curve. Use the OD value of the sample to determine the corresponding concentration from the standard curve, and then multiply by the dilution factor. Alternatively, calculate the linear regression equation of the standard curve using the concentrations and OD values of the standards. Substitute the sample's OD value into this equation to calculate the sample concentration, and then multiply by the dilution factor to obtain the actual concentration of the sample.

Detection Range

10 pg/ml-700 pg/ml

Precautions

1. The kit should be equilibrated at room temperature for 1 hour after being removed from refrigeration before use. If the enzyme-coated plates are not fully utilized after opening, any unused strips should be stored in a sealed bag.
2. Concentrated wash solution may crystallize. If this occurs, warming the solution in a water bath can assist with dissolution without affecting the washing results.
3. A pipette should be used for each step of sample addition, and its accuracy should be regularly verified to avoid experimental errors. It is best to complete sample addition within 5 minutes. If the number of samples is high, it is recommended to use a multichannel pipette.
4. A standard curve should be created with each assay, ideally with duplicate wells. If the concentration of the analyte in the samples is too high (i.e., the sample OD value exceeds that of the first standard well), dilute the sample with dilution solution by a certain factor (n times) before measurement. When calculating, multiply by the total dilution factor (×n×5).
5. The sealing film is for single-use only to prevent cross-contamination.
6. Store the substrate protected from light.
7. Follow the instructions strictly during operation, and the judgment of experimental results must be based on the readings from the ELISA reader.
8. All samples, wash solutions, and various waste materials should be disposed of as infectious substances.

9. Components from different batches of this reagent must not be mixed.

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