EQUINE TSH

EQUINE THYROID STIMULATING HORMONE (TSH) ELISA TEST

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

The Equine TSH ELISA test is an immunoassay designed for the quantitative determination of thyroid stimulating hormone (TSH) in serum/plasma samples of Equine and related species. The test is intended for professional use as an aid in the diagnosis and monitoring of physiological/pathological conditions related to circulating TSH in equine and related species.

TEST PRINCIPLE:

The Equine TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes affinity purified antibody directed against intact equine TSH molecule for solid phase (microtiter wells) immobilization and a mouse anti-TSH antibody is in the enzyme (horseradish peroxidase) conjugate. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 2 hour incubation period at 37ºC, the wells are washed with wash solution to remove unbound antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution resulting in yellow color, which is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is directly related to the amount of TSH in the sample. A series of TSH standards assayed in the same way, a standard curve is constructed and the concentration of TSH in the unknown sample is quantified.

REAGENTS AND MATERIALS PROVIDED:

1. Antibody-coated microtiter plate (96 wells)
2. Reference standards 150, 50, 25, 10, 5, 1, 0 ng/ml (0.3ml/vial)
3. HRP-Enzyme Conjugate, 12mL
4. TMB color reagent, 12mL
5. Wash Buffer, 20 X, 20 mL
6. Stop solution (2N HCl), 6mL
7. Sample/Standard diluent, 10mL

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Vortex mixer or equivalent
4. Absorbent paper of paper towel
5. Graph paper
6. Microtiter plate reader

SPECIMEN COLLECTION AND PREPAEQUINEION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum/plasma samples only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8^{O}C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement. **Call Endocrine for your ELISA reader requirements.**

REAGENT PREPARATION

1. All reagents should be brought to room temperature (25-28^{O}C) before use.
2. The diluted standards are good for one week, when stored at 2-8^{O}C. If not used for a long time, should be kept frozen at -20C.
3. Dilute wash buffer from stock 1part with 9 parts of distilled water.

ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results.

Use clean pipettes and sterile, disposable tips:

1. Secure the desired number of coated wells in the holder.
2. Dispense 100ul of standards, specimens, and controls into appropriate wells.
3. Dispense 100ul of Enzyme Conjugate into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing at this step.
5. Incubate at 37ºC for 2 hours in a sealed container or use zip-lock bag.
6. Remove the incubation mixture by dumping plate contents into a waste container.
7. Rinse and dump the microtiter wells five (5) times with diluted wash buffer.
8. Strike wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100ul of TMB solution into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop reaction by adding 50ul of stop solution, 2N HCl to each well.
12. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
13. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical. Insufficient washing will result in poor precision and falsely elevated absorbence readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and test samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbency values on the vertical or Y axis, and concentration on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of TSH in ng/ml from the standard curve.

EXPECTED VALUES AND SENSITIVITY

The minimal detectable concentration of Equine TSH by this assay is estimated to be 0.5 ng/ml. and the normal and experimental values should be established in your own laboratory. Each laboratory must follow good laboratory practice and maintain proper documentation.

REFERENCES

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