FELINE TSH

FELINE THYROID STIMULATING HORMONE (TSH) ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

The Feline TSH ELISA test is an immunoassay designed for the quantitative determination of thyroid stimulating hormone (TSH) in serum/plasma samples of Feline and related species.

TEST PRINCIPLE:
The Feline 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 human TSH molecule for solid phase (microtiter wells) immobilization and a goat anti-TSH antibody is in the antibody-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 3hours of incubation period at 37ºC, the wells are washed with wash solution to remove unbound-labeled 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 and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED:
1. Antibody-coated microtiter wells
2. Reference standard, Ready to use (0, 1, 2.5, 5.0, 10, 25 ng/mL)
3. Enzyme Conjugate Reagent, 12 mL
4. TMB color reagent (ready to use), 12 mL
5. 20X Wash buffer, 20 mL
6. Stop solution (2N HCl), 6mL
7. Sample/Standard diluent, 10mL
8. Instructions

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 PREPACANINEION
Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum/plasma samples only. The sample containing media should be further diluted with Sample/standard dilution buffer (SSD) before using in the assay system.

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 expire 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 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPACANINEION
1. All reagents should be brought to room temperature (25-28^{O}C) before use.
2. Reconstitute lyophilized standard with 1.0mL of sample/standard diluent (SSD). Allow the reconstituted material to stand for at least 20 minutes.
3. The standards should be kept frozen at -20C if not used immediately.
4. Dilute wash buffer, desire amount with distilled water (1part with 19 parts). The buffer is stable for 1-3 months, if stored at 4-8C.

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 Reagent 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 3hours in a sealed container or use zip-lock bag (provided).
6. Remove the incubation mixture by decanting the plate contents into a waste container.
7. Rinse and decant the microtiter wells five (5) times with wash buffer.
8. Strike the 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 (one drop) 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 step is 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 Feline TSH by this assay is estimated to be 1.0 ng/ml,
the normal and experimental values should be established in your own laboratory. Each lab must follow good lab practice and maintain proper documentation.

REFERENCES

1. Graves TK, Peterson ME. Diagnostic tests for feline hyperthyroidism Vet Clin North Am Small Anim Pract 1994 May;24(3):567-76
2. Brown RS, Keating P, Livingston PG, Bullock L. Thyroid growth immunoglobulins in feline hyperthyroidism.Thyroid. 1992 Summer;2(2):125-30.
3. Peterson ME, Kintzer PP, Cavanagh PG, Fox PR, Ferguson DC, Johnson GF, Becker DV. Feline hyperthyroidism: pretreatment clinical and laboratory evaluation of 131 cases. J Am Vet Med Assoc. 1983 Jul 1;183(1):103-10.
4. Peterson ME, Melian C, Nichols R Measurement of serum concentrations of free thyroxine, total thyroxine, and total triiodothyronine in cats with hyperthyroidism and cats with nonthyroidal diseaseJ Am Vet Med Assoc 2001 Feb 15;218(4):529-36
5. Segal J, Troen BR, Ingbar SH. Influence of age and sex on the concentrations of thyroid hormone in serum in the rat. J Endocrinol 1982 May;93(2):177-81
6. Walker W.H.C. Introduction: An Approach to Immunoassay. Clin. Chem. 1977; 23: 384
7. Wisdom G.B. Enzyme-Immunoassay. Clin. Chem. 1976; 22: 1243
8. Hoffenberg R. Medicine 1978; 8: 392
9. Lieblich J., Utiger R.D. J. Clin. Invest. 1972; 51: 1939

Revised 012010

www.endocrinetech.com

35325 Fircrest Street, Newark, CA 94560-1003 * Phone (800) 745-0843 * (510) 745-0844 * Fax (510) 745-0977

Quality Control Data:
It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired age, sex and physiological parameters.
A typical standard curve (illustration only) for Feline TSH is given below:

Standard ng/mL	**OD at 450nm**
0	**0.09**
1.0	**0.24**
2.5	**0.67**
5	**0.92**
10	**1.64**
25	**282**

ELISA Performance Characters
Precision: Inter and Intra assay variation (CV) was determined from three different pooled serum samples in three different experiments.

Inter-assay variation	Set1: CV= 6.6 % (N=10)	Set2: CV= 7.8%(N=10)	Set3: CV= 6.4% (N=10)
Intra-assay variation	Set1: CV= 8.7 (N=10)	Set2: CV= 8.6%(N=10)	Set3: CV= 8.8% (N=10)

Sensitivity: The lowest level detectable in this assay is 0.5g/mL of serum or plasma

Specificity: The Feline TSH ELISA system utilizes monoclonal antibody and high affinity polyclonal antibody to TSH. The cross reactivity to other pituitary gonadotropins (Feline LH, FSH) is not detectable under the conditions of the assay system.

Distributed by:
Xceltis
Xceltis GmbH
Im Tal 12
74909 Meckesheim / Germany
Tel.: +49-6226-9724-18
Fax: +49-6226-9724-19
E-mail: info@xceltis.de