SKU IP700002

FELINE TSH ELISA

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

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VERSION 1.3

INTENDED USE

To detect Feline Thyroid Stimulating Hormone (TSH) in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standards A to F [0, 0.75, 1.5, 3, 6, 12mU/L]
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]
Zipper bag [1pc]

MATERIALS REQUIRED BUT NOT INCLUDED

Distilled Water, Pipettes and Tips, ELISA Reader 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, Cell Culture Supernatants, Urine, Saliva Biological Fluids, etc.

Detection Range: 0.5mU/L - 12mU/L
Minimum Detection Dose: 0.01mU/L
Intra-Assay Precision: CV<9%
Inter Assay Precision: CV<11%
Linearity with spiked analytes: ρ=0.99
Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacture
Storage: 2-8°C

Once Opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

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. The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue. 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 5000×g to collect supernatant.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles. Note: Samples should be centrifuged adequately and no hemolysis or granule was 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 Testing wells and add 10µl samples to the testing sample wells, then add 40µl sample diluent to the testing sample wells. In Blank wells do not add anything.

3. Add 100µl of HRP-conjugate reagent to each well (Standard wells and Testing 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. Repeat 5 times. 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 (e.g. it is 5 in this case).

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.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 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.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- 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.

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