Canine TSH ELISA Kit

Enzyme Immunoassay for the quantification of Thyroid stimulating hormone (TSH) in canine serum or plasma.

Catalog number: ARG80663

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Thyroid stimulating hormone (TSH, thyrotropin) in dogs is similar in function to TSH found in other mammalian species, including humans. It is a glycoprotein produced by the anterior pituitary gland. Through its action on the thyroid gland, it plays a major role in maintaining normal circulating levels of the iodothyronines, T4 and T3. The production and secretion of TSH is controlled by negative feedback from circulating T4 and T3, and by the hypothalamic hormone TRH (thyrotropin releasing hormone).

The TSH molecule is composed of two nonidentical subunits, α and β, that are bound together in a noncovalent manner. Within a species, the TSH α subunit is structurally identical to the α subunits of the related glycoprotein hormones (LH, FSH and chorionic gonadotropin). The β subunit of TSH and the β subunits of the related hormones are structurally hormone-specific, and confer upon them their unique biological activities. Hypothyroidism is considered to be a common endocrine disorder in dogs, whereas hyperthyroidism in this species is nearly unknown. Most cases of canine hypothyroidism are primary in nature, involving impaired production of the thyroid hormones, T4 and T3. In this condition, elevated TSH levels are expected. Secondary or tertiary hypothyroidism, where thyroid hormone production is low as a consequence of hypothalamic or pituitary disease, is believed to account for less than 5% of canine hypothyroidism cases.

In the latter conditions, lowered levels of TSH would be expected. Usually,

hypothyroidism in dogs is suspected on the basis of clinical history and the presence of lowered levels of thyroid hormones. However, suppressed thyroid hormone levels are nonspecific indicators of the disease, since they are often observed in nonthyroid illnesses. The evaluation of thyroid function and the diagnosis of hypothyroidism in dogs can be greatly improved through the use of the valid assay for the determination of canine TSH.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for canine TSH has to be bound onto a pre-coated microtiter plate. Standards or samples are pipetted into the wells and any canine TSH present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for canine TSH is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of canine TSH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of canine TSH in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C.
Canine TSH Calibrators	6 vial (0, 0.2, 0.46, 1.05, 2.2 and 5.2 ng/ml)	4°C, lyophilized
HRP conjugated antibody	11 ml (ready for use)	4°C
10X Wash Buffer	50 ml (ready for use)	4°C
TMB substrate	22 ml	4°C (Protect from light)
STOP solution	7 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Calibrators: Reconstitute lyophilized Calibrators A through F with 1.0 ml distilled water 30 minutes before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into sample wells.
3. Add 100 µl of HRP conjugated antibody into each well. Incubate for 2 hours at RT.
4. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 200 µl of TMB mixture to each well. Incubate for 30 minutes at room temperature in dark.
6. Add 50 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

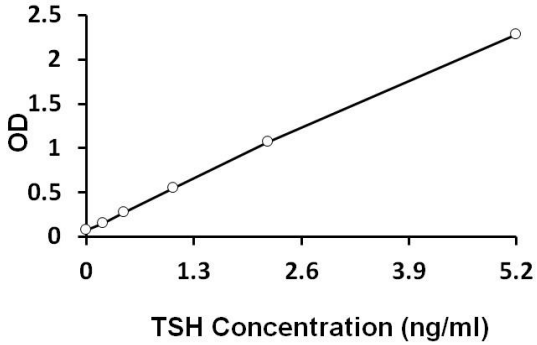
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The assay's detection limit, defined as the concentration two standard deviations above the response at zero dose, is approximately 0.01 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.33% and inter-assay precision was 6.45%.

Recovery

95-101%