Canine Thyroxine (T4) ELISA kit

Catalog No.E0452c

(96 tests)

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FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Intended use

This immunoassay kit allows for the specific measurement of total T4 concentrations of natural and recombinant canine T4 in plasma and serum.

Introduction

Thyroxine (T4), the principal thyroid hormone largely bound to transport proteins, especially TBG. Given normal levels of thyroid hormone-binding proteins, hyperthyroidism is characterized by increased levels of circulating T4, hypothyroidism by decreased levels. Exceptions to this parallelism between thyroid status and total T4 concentration are found. Levels of TBG are known to be altered under various physiological, pharmacological and genetic conditions. Thus, elevated T4 levels may be obtained when TBG levels are high, as in pregnancy, acute intermittent porphyria, hyperproteinemia, hereditary TBG elevation and in patients undergoing estrogen therapy or taking oral contraceptives. Total T4 levels may be depressed when TBG levels are low, as in nephrotic, hepatic, gastrointestinal and neoplastic disorders; in acromegaly, hypoproteinemia and hereditary TBG deficiency; and in patients undergoing androgen, testosterone or anabolic steroid therapy. Diphenylhydantoin and large doses of salicylates and liothyronine may also cause low T4 values (not reflective of thyroid status) due to their competition fo binding sites on TBG.

Test principle

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific for T4 has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled T4 and biotin unlabeled antigens (Standards or samples) with the pre-coated antibody specific for T4. The more the amount of T4 in samples, the less the biotin labeled T4 bound by pre-coated antibody. Vice versa. Then HRP labeld avidin and substrate solution are added to the wells, respectively. And the color develops in opposite to the amount of T4 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Materials and components

Reagent	Quantity
assay plate	1
standard	6×1 ml
Detection Reagent A	1×10ml
Detection Reagent B	1×10 ml

Wash Buffer (25 x concentrate)	1×30ml
Substrate Solution A/B	2×10 ml
Stop Solution	1×20 ml

Limitations of the procedure

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1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Sample collection and storage

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

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8° C within 30 minutes of collection. Store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

Reagent preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

T4 Standard – Concentration of T4 standard that the kit provided is 400 ng/mL, 200 ng /mL,100 ng /mL, 50 ng /mL, 25ng /mL and 0 ng/mL.

Assay procedure

Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Add 50 μL of Standard, Control, or sample per well. And then add 50 μL of Detection Reagent A. Incubate for 1 hour at 37° C.
3. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 50 μL of T4 Detection Reagent B to each well. Incubate for 30 minutes at 37° C .
5. Repeat the aspiration/wash as in step 4.
6. Add 50 μL of Substrate Solution A and B to each well, respectively. Incubate for 15 minutes at 37° C. Protect from light.
7. Add 50 μL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and natural canine T4. No significant cross-reactivity or interference was observed.

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. 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.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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 mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the T4 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.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.