

Canine T3 ELISA Kit

to quantitatively determine Canine T3 in Canine Blood Serum or Plasma

INSTRUCTION MANUAL

FOR ELISA KIT No: LT05406CETKKBA



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INTENDED USE

This kit is used to quantitatively determine the Canine T3 in the sample of Canine Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X2

BACKGROUND

Thyroid hormones thyroxin (T4) and 3,5,3'-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4. The concentration of T3 is much less than that of T4 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of T4, and only 20% is secreted by thyroid gland. That is why in hypothyroid patients T3 level may for a long time remain on the lower limit of the normal range, because its loss may be compensated by enhanced conversion of T4 into T3. Determination of T3 level is most useful in T3-hyperthyroidism because 5-10% of such patients do not show significant changes in T4 level while concentration of T3 is highly elevated. Elevated T3 levels are seen in early thyroid hypofunction, after intake of estrogens, oral contraceptives, heroin, methadone, during pregnancy. Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salicylates.

PRINCIPLE

This test is based on competition enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific rabbit polyclonal to T3-antibodies simultaneously with conjugated T3-peroxidase. T3 from the specimen competes with the conjugated T3 for coating antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and

quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is inversely related to the quantity of the measured analyte in the specimen.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator 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.
- 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.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Incubator
2. Microplate reader
3. Precision pipettes and tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8°C. Immediately after use remaining reagents should be returned to cold storage at 4°C

MATERIALS SUPPLIED IN THIS KIT

1. T3 Strip Plates, 8 X 12 wells. Qty: 1
2. Calibrators: C1: 0, C2: 0.75, C3: 1.5, C4: 7.5, C5: 15 nmol/l, 5pcs
3. Control Serum, Qty: 1
4. Conjugate Concentrate 2X, Qty: 1
5. Conjugate Dilution Buffer, Qty: 1
6. Substrate Solution, Qty: 1
7. Wash Buffer Concentrate 26X, Qty: 1
8. Stop Solution, Qty: 1
9. Plate Sealing Tape, Qty: 2
10. Instruction Manual, Qty: 1

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. 2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. 3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation

steps is necessary.

- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2...+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided. 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 and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw

cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 °C. If samples are to be used within 24 hours, they may be stored at 2 to 8 °C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

—All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18...+25 °C) before use.

—All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

—It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

—Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

—It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

— Note alternative unit: 1 nmol/l = 0.65 ng/ml

ASSAY PROCEDURE

1 Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. **DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.**

2 Prepare working conjugate solution by dilution of conjugate concentrate 2 fold

by conjugate dilution buffer.

ATTENTION: working conjugate solution is unstable and should not be stored!

Prepare the volume required for actual assay run.

3 Pipet 25 µl of calibrators CAL 1–5, control samples CONTROL and unknown samples into the wells.

4 Dispense 100 µl of working conjugate solution into the wells. Cover the wells by plate adhesive tape (included into the kit).

5 Incubate 60 minutes at 37 °C.

6 Prepare washing solution by 26x dilution of washing solution concentrate (BUF WASH 26X) by distilled water.

Wash the strips 5 times.

7 Dispense 100 µl of SUBS TMB into the wells

8 Incubate 10–20 minutes at +18...+25 °C

9 Dispense 100 µl of STOP into the wells.

10 Measure OD (optical density) at 450 nm.

11 Set photometer blank on air

12 Apply lin-log method for data reduction.

PERFORMANCE CHARACTERISTICS

Analytical specificity / Cross reactivity

L-T3: 100 [Analyte Cross-reactivity, % wt/wt]

D-T3: 100 [Analyte Cross-reactivity, % wt/wt]

L-Thyroxin: 0.01 [Analyte Cross-reactivity, % wt/wt]

D-Thyroxin: 0.04 [Analyte Cross-reactivity, % wt/wt]

Sensitivity of the assay was assessed as being 0.2 nmol/l.

Linearity was checked by assaying dilution series of 5 samples with different triiodothyronine concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known triiodothyronine concentrations. The recovery percentages ranged from 90 to 110%

CALCULATION

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples. Plot a calibration curve on graph paper: OD versus triiodothyronine concentration. Determine the corresponding concentration of triiodothyronine in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

VALIDITY & STORAGE: 12 months (at 2-8°C, unopened).