



# CANINE T4

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## CANINE THYROXINE (T4) ELISA TEST KIT

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### PRODUCT PROFILE AND INSTRUCTIONS

The T4 ELISA test is an immunoassay designed for the quantitative determination of thyroxine (T4) in serum/plasma samples of canine and related species.

#### TEST PRINCIPLE

In the T4 ELISA Test, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of unknown sample and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the T4 and conjugated T4 compete for the limited binding sites on the anti-T4 antibody. After 60 minutes of incubation period, at 37°C, the wells are washed 5 times with wash buffer to remove any unbound T4 conjugate. A solution of TMB is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T4 in the sample. A series of T4 standards assayed in the same way, a standard curve is constructed and the concentration of T4 in the unknown sample is quantified.

#### MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Reference Standard, 1 set, ready to use (0.5, 10, 25, 50, 100, 300 ng/mL)
3. T4 HRP Conjugate Reagent, 12 mL
4. TMB Color Reagent (ready to use) 12 mL
5. Stop solution (2N HCl) 6mL
6. 20X Wash buffer, 20 mL
7. 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 or paper towel
5. Graph paper
6. Microtiter plate reader

#### SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum/plasma samples only.

#### STORAGE OF TEST KIT AND INSTRUMENTATION

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. Opened test kits will remain stable until the expiration 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 PREPARATION & SAMPLES

1. All reagents should be brought to room temperature (25-28°C) before use.
2. Must read and follow instructions carefully and any deviations may result in poor sensitivity or high background.
3. Prepare desired amount of wash buffer by diluting 1 part with 19 parts of distilled water. This buffer may be stored at 4-8°C for 1-3 months.
4. Reference standards are ready to use liquids and should be stored at -20°C, if not used immediately.



## 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 50ul of standards and specimens into appropriate wells.
3. Dispense 100ul of T4 HRP-Conjugate Reagent into each well. Mix for 30 seconds. It is very important to have complete mixing at this step.
4. Incubate at 37°C for 60 minutes.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100ul of TMB color reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50ul of 2N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450nm with a microtiter well reader.

*Important note:* The wash step is very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

## CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient 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 concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of T4 in ng/mL from the standard curve.

## EXPECTED VALUES AND SENSITIVITY

It is recommended that each laboratory should establish values to reflect differences specific to experimental conditions. The minimum detectable concentration of thyroxine by this assay is estimated to be 1 ng/mL.

## REFERENCES

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**CANINE T4 ELISA Test Kit**

**Professional use only**

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

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