DRG® Total T4 (Canine) ELISA (EIA-2492)

REVISED 28 FEB. 2011 RM (VERS. 5.1) **FOR VETERINARY USE ONLY**

1 INTRODUCTION

Dogs suffering from reproductive dysfunction, poor coat, unexplained lethargy, obesity, hyperlipidemia, myopathy, megaesophagus and failure to grow should be tested for T4 total concentrations. Up to 20% of normal dogs have decreased serum/plasma T3/T4-total levels (Muller et all '83'). T4 total levels decrease during aging and certain breeds, C. Spaniel, Labrador, Malamute Husky, have lower T4 total levels.

Other clinical parameters, which are usually influenced, are:

Increased: - GPT (ALAT), ASP, LDH, GOT (ASAT).
Decreased: - Lymphocytes

2 INTENDED USE OF THE TEST KIT

The Canine T4 total ELISA is designed to detect T4 total in individual serum and plasma samples.

For this purpose monoclonal anti-T4 total antibodies attached to the plate will catch the thyroxin in the sample to be tested. The thyroxin present in the sample will compete with the specific biotin-marked thyroxin conjugate. After incubation the ELISA will be washed to remove unbound thyroxin. Peroxidase marked streptavidin conjugate will be added to the ELISA wells. After incubation the ELISA will be washed to remove unbound streptavidin. Substrate will be added to the ELISA wells and the color development is directly correlated with the quantity of bound thyroxin.

3 PRINCIPLE OF THE TEST KIT

The test is based on the competition of thyroxin in the sample to be tested, with known biotin marked thyroxin conjugate. To this end monoclonal anti-T4 total antibodies are coated to a 96-well microtiter strip plate.

The canine serum/plasma sample is added together with the biotin marked T4 to the wells of the coated plate.

Color reaction in the wells is inversely directly related to the concentration of thyroxin in the serum/plasma sample.

4 CONTENTS

- 12 x 8 **microtiter strips**.
- 1 x **Strip holder**.
- 1 x 7 mL T4 **ELISA Buffer**.
- 1 x 7 mL **biotin marked thyroxin Conjugate**.
- 1 x 12 mL **Conjugate Buffer**
- 1 x 0.2 mL 100X concentrated **Streptavidin Conjugate**
- 1 x **Standard 1** (0 nmol/L).
- 1 x **Standard 2** (50 nmol/L).
- 1 x **Standard 3** (100 nmol/L).
- 1 x **Standard 4** (250 nmol/L).
- 1 x 20 mL **Wash Solution** (200X concentrated), **dilute in deionized water before use!**

- 1 x 8 mL **Substrate A**.
- 1 x 8 mL **Substrate B**.
- 1 x 8 mL **Stop Solution**.
- 1 x Plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at +4 °C.

An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20 °C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity.

6 WASH PROTOCOL

In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µL washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times
4. Turn the plate upside down and empty the wells with a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL

1. Open the packet of strips and only take out the strips to be used.
   A minimum of 5 wells is required for the 4 standards and 1 for the serum/plasma sample.
   Cover the remaining strips with the provided seal and store them in the box at 4 °C – 8 °C.
   Take out the T4 ELISA buffer, the standards and the biotin marked thyroxin conjugate. Bring all reagents to room temperature. Minimize repeated heating and cooling of the ELISA kit because this will influence the quality of the ELISA kit.
2. Add 60 µL of ready to use T4 ELISA buffer to all wells to be used.
3. Add 50 µL of standard to each of the consecutive wells.
4. Add 50 µL of sample(s) to the next well(s).
5. Add 60 µL of ready to use biotin marked thyroxin conjugate to all wells.
6. Gently tap the wells to mix all reagents.
7. Cover the strips and incubate 60 minutes at 21 °C (room temperature)
8. Wash the strip(s) with washing solution; according to washing protocol (see Chapter 6).
   Dilute the wash solution 200X in de-ionized water before use!
9. **Dilute the 100X concentrated streptavidin-conjugate 1:100 in the provided conjugate buffer**
10. Add 100 µL of the diluted peroxidase marked streptavidin conjugate to all wells.
11. Gently tap the wells to mix all reagents.
12. Cover the strips and incubate 30 minutes at 21 °C
13. Wash the strip(s) with washing solution; according to washing protocol (see Chapter 6).
    Dilute the wash solution 200X in de-ionized water before use!
14. Prepare the substrate solution immediately before use!
    Mix equal parts of substrate buffer A and substrate buffer B while gentle mixing.
15. Add 100 µL of substrate solution to each well. Protect the strips from direct light (Example: place a piece of clean paper on top)
    and incubate 10- 13 minutes at room temperature (21 °C).
16. Add 50 µL of stop solution to each well; mix carefully by gently tapping the wells. Make sure that all blue coloration has changed into yellow.
17. Read the absorbency values immediately (within 10 minutes!) at 450 nm.

8 PRECAUTIONS

- Handle all biological materials as though capable of transmitting diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.

- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

9 INTERPRETATION OF TEST RESULTS

All results should be placed in a graphic to determine the concentration of thyroxin in the unknown specimen(s).

1. Record the absorbency obtained from the printout of the microplate reader.
2. Plot the absorbency for each standard versus the corresponding thyroxin concentration in nmol/L on linear graph paper.
3. Draw the best-fit curve through the plotted points.

To determine the concentration of thyroxin for an unknown specimen, locate the unknown on the vertical axis of the graph, find the intersecting point on the curve and read the concentration (in nmol/L) from the horizontal axis of the graph.

Standards:	-	Canine: 19 – 58 nmol/L
Increased:	-	Hyperthyroidism
	-	Thyroxin overdose
	-	TSH stimulation
	-	Oestrus
Decreased:	-	Hypothyroidism
	-	Fat Mobilization Syndrome, Ketosis
	-	Hyperadrenocorticism

Remarks : Treatment with Androgens, corticosteroids, diazepam, high doses of iodine, mithodane, penicillin, phenobarbital, phenylbutazon, primidon, propylthiourazil, salysilate, may cause decrease of the thyroxin levels

The entire risk as to the performance of these products is assumed by the purchaser. DRG shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.