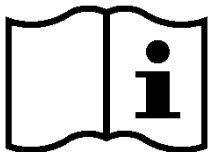

Userś Manual

Canine T4 Total

ELISA

An ELISA to detect the total T4 concentration in serum and plasma samples of dogs

REF D1010-HR01

96

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Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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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 total T4 in individual serum and plasma samples. For this purpose monoclonal anti-T4 total antibodies attached to the plate will catch the bound and unbound thyroxin (T4) 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 streptavidine conjugate will be added to the ELISA wells. After incubation the ELISA will be washed to remove unbound streptavidine. Substrate will be added to the ELISA wells and the color development is inversely related 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 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 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 (200xconcentrated), **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
- 1 x User's manual

Supplies needed (not included)

- Validated precision pipette
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

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.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

6. Wash protocol

In ELISA's, 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 result.

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 wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 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 wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol qualitative

Before starting this test read "preparations"

1. Take out the ELISA buffer, standards and biotin marked thyroxin conjugate and bring all reagents at room temperature.
2. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C and use them within 10 days.

   Wash microtiter strip(s) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water!

3. Use the validated precision pipette and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

4. Add **60 µl** of ready to use **T4 ELISA buffer** to all wells to be used.
5. Add **60 µl** of **standards** to each of the consecutive wells.
6. Add **60 µl** of **sample**(s) to the next well(s).
7. Add **60 µl of biotin marked thyroxin conjugate** to all wells.
8. Gently tap the wells to mix all reagents.
9. Cover the strips and incubate 60 minutes at 21°C (room temperature).

10. Wash the microtiter strip(s) with washing solution, according to the washing protocol.

11. Add **100µl of the peroxidase marked streptavidine conjugate** to all wells.
12. Gently tap the wells to mix all reagents.
13. Seal and incubate for 20 min at room temperature.

14. Wash the plate according to the wash protocol see sub 6.
15. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**

16. Add **100µl substrate solution** to each well.
17. Incubate 6-8 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative does not become too dark.

18. Add **50µl stop solution** to each well; mix well.
19. Read the absorbency values immediately (within 10 min!) at 450 nm using 620nm as reference on the ELISA reader.

9. Precautions

- Handle all biological material as though capable of transmitting infectious 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 B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling 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.

10. Interpretation of the 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 microtiter plate 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

11. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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

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