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User's Manual

T4 total feline ELISA

A rapid Enzyme immunoassay to measure the total T4 concentration in serum and plasma samples from feline species.

VET

REF

DE3442



32

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1 INTRODUCTION

For Veterinary use only!

Cats suffering from T4 problems can mostly be recognized on the following symptoms: weight loss, polyphagia, vomiting, polyuria, polydipsia, hyperactivity, cardiac disease, large faeces volumes.

These are all mainly indicators of hyperthyroidism. Despite these symptoms, cats still stay active and do not lose their appetite. In most cases 80% enlargement of one or both thyroid lobes can be detected. Spontaneous feline hyperthyroidism may also exist and cause coetaneous abnormalities.

Serum T4 and T3 levels are decreased by many drugs (androgens, heparin, glucocorticoids, phenothiazines, etc.). Also estrus and pregnancy can increase T3 and T4 concentrations. Fasting does not affect serum levels, stable for 8 days at room temperature. After treatment, blood concentration peak appears between 6 -8 hours post-treatment.

Other clinical parameters which are usually influenced are:

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

2 INTENDED USE AND PRINCIPLE OF THE TEST

The T4 (Feline) Rapid 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 plate will be washed to remove unbound thyroxin.

Peroxidase marked streptavidin conjugate will be added to the wells. After incubation the ELISA will be washed to remove unbound streptavidin. Substrate will be added and the colour development is inversely proportional to the concentration of thyroxin in the sample.

3 CONTENTS

- 4 x 8 well, **Test Strips** (32 wells)
- 1 x **Standard 1**, 0 nmol/L (green)
- 1 x **Standard 2**, 50 nmol/L (brown)
- 1 x **Standard 3**, 100 nmol/L (red)
- 1 x **Standard 4**, 250 nmol/L (yellow)
- 1 x **Buffer** (bottle + green cap)
- 1 x **Biotin Conjugate** (white bottle + black cap)
- 1 x **Streptavidin Conjugate** (black bottle + red cap)
- 1 x **Substrate A** (bottle + white cap)
- 1 x **Substrate B** (bottle + blue cap)

3.1 Supplies needed (not included)

Precision pipette 10 - 200 µL

Pipette tips

Optional: A microtiter plate reader (The results can be interpreted by eye, but for a more accurate and objective reading a microtiter plate reader can be used)

4 HANDLING AND STORAGE OF REAGENTS AND 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.

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5 PREPARATIONS

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 minutes at room temperature (21 °C) without exposing them to direct sunlight or (other) heat sources.
- Buffers, 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 2 °C - 8 °C immediately after use.

6 TEST PROTOCOL

1. Before starting this test read "*Preparations*"
2. Break the amount of wells needed from the test strip, 1 for each sample and 4 extra wells for the standards. Use the Precision pipette 10 - 200 μ L and use a clean pipette tip **before** pipetting the buffer, standards, samples, conjugate and substrate.
3. Before testing make sure all reagents are at room temperature
4. Add 60 μ L of buffer to each well. (see fig. 1)
5. Add 50 μ L of Standard 1, 0 nmol/L to the first well. (see fig. 2)
6. Add 50 μ L of Standard 2, 50 nmol/L to the second well.
7. Add 50 μ L of Standard 3, 100 nmol/L to the third well.
8. Add 50 μ L of Standard 4, 250 nmol/L to the fourth well.
9. Add 50 μ L of Sample (serum/ plasma) to the remaining wells.
10. Add 60 μ L of Biotin Conjugate to each well. (see fig. 3)
11. Mix the reagents gently (see "*Preparations*").
12. **Incubate 60 minutes** at room temperature (± 21 °C).
13. Wash the test strips with running tap water: Fill all wells to the rim. Empty the wells. Repeat 5 times. Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed. (see fig. 4)
14. Add 100 μ L of the Streptavidin Conjugate to each well and **incubate 25 minutes**. (see fig. 5)
15. Wash as in 13.
16. Add 60 μ L of substrate A to each well. (see fig. 6)
17. Add 60 μ L of substrate B to each well.
18. Mix the reagents gently (see "*Preparations*").
19. **Incubate for 7 – 10 minutes in the dark** (e.g. cover the wells with a sheet of paper)
20. Mix the reagents gently (see "*Preparations*").
21. Read the results by eye
or
using a microtiter plate reader with 620/630 nm filter (reference wavelength 450 nm). If a plate reader is used, the reading must be done immediately.
(See "*Interpretation of results*".)

7 PRECAUTIONS

- Handle all biological materials as though capable of transmitting human pathogens.
- 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.

8 INTERPRETATION OF RESULTS

The microtiter plate reader will give the results nmol/L, but always double-check the outcome by observing the intensity of colour development.

Visual Interpretation

The T4 concentration in the samples can be determined by relating them to the standards.

The degree of colour development is inversely proportional to the T4 concentration.

Colour	T4 level	Result
Dark blue	<18 nmol/L	T4 is too low
Blue	19 - 65 nmol/L	T4 is normal
Light blue	65- 100 nmol/L	T4 is a bit to high
Clear blue	>100 nmol/L	T4 is too high

For example (see fig. 8): The colour of the sample corresponds with the third well.

To the third well, 100 nmol/L has been added, therefore the sample also contains ± 100 nmol/L.

Interpretation with a microtiter plate reader

Using graph paper, construct a standard curve by plotting the optical density (OD) obtained from each standard against its concentration (in nmol/L) with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.

Using the OD value for each sample determine the corresponding concentration from the standard curve.

This can also be done using appropriate software.

Note: These results are only an indication. The final diagnosis shall have to be made by the Veterinarian on the basis of this result and available clinical information.

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

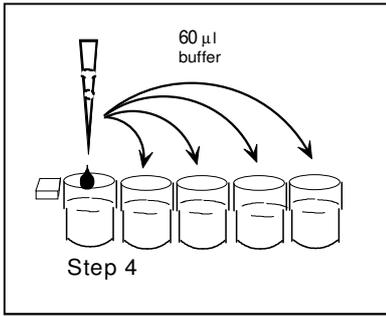


Fig. 1 Buffer procedure

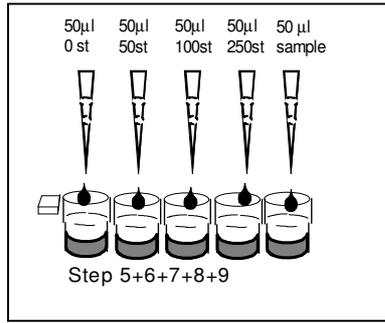


Fig. 2 Sampling procedure

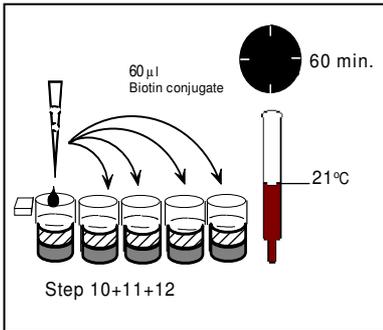


Fig. 3 Biotin Conjugate procedure

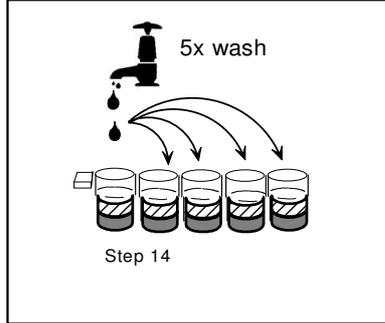


Fig. 4 Wash procedure

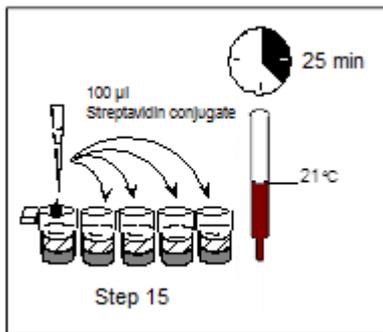


Fig. 5 Streptavidin Conjugate procedure

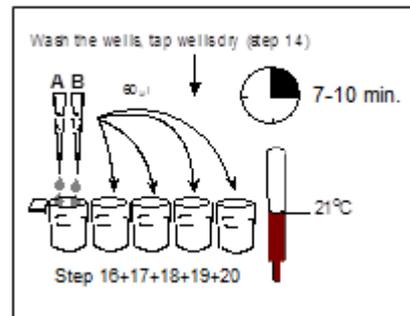


Fig. 6 Substrate procedure

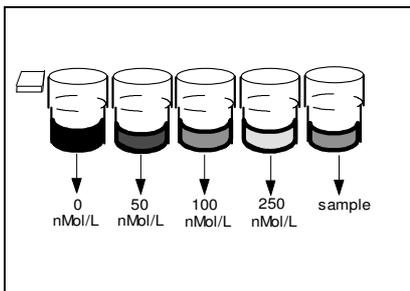


Fig. 8 Example result

SYMBOLS USED WITH DEMEDITEC 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
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For veterinary use only				
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Mindesthaltbarkeitsdatum	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