DRG® T4 (Feline) Rapid ELISA (EIA-3442)

Revised 28 Feb. 2011 rm (Vers. 3.1) **For Veterinary Use Only**

Please use only the valid version of the package insert provided with the kit.

1 INTRODUCTION

Cats suffering from reproductive dysfunction, poor coat, unexplained lethargy, obesity, hyperlipaedemia, myopathy, megaesophagus and failure to grow should be tested for T4 total concentrations.

Up to 15% of normal Cats have serum/plasma T4 total levels out of standard range T4 total levels decrease during ageing and certain breeds have lowerT4 total levels.

Other clinical parameters which are usually influenced are:

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

2 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 buffer (black bottle + red cap)

1 x concentrated Streptavidin conjugate (dilute 1:100)

1 x substrate A (bottle + white cap)

1 x substrate B (bottle + blue cap)

3 SUPPLIES NEEDED (NOT INCLUDED)

Precision pipette 10-200 µl

Pipette tips

Vet Diagnostic Analyser (The results can be interpreted by eye, but for a more accurate and objective reading the use of the Vet Diagnostic analyser is strongly recommended)

4 STORAGE

This product has a limited shelf life (see "expiry date"). The expiration date is only warranted when all components have been stored at 4°- 8°C and in a sealed package.

Always close the package well after using the products.

5 PRECAUTIONS

- Handle all biological materials as though capable of transmitting human pathogens.
- Do not eat, drink, smoke, prepare food, or apply cosmetics in the working area.
- TMB is toxic by inhalation, through contact of the skin or when swallowed.
- Observe care when handling the substrate.
- Do not mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing during this procedure is necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore do not touch the undersurface of the microtitre plate and prevent it from getting damage or dirty.
- Stop solution contains acid, observe care when handling the stop solution.

6 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.
- 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 resuspend 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.

7 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 controls. Use the Precision pipette 10-200 µl and use a clean pipette tip **before** pipetting the buffer, standards, samples, diluted conjugate and substrate.
3. Before testing make sure all reagents are at room temperature
4. Add 60 µl of buffer to each well. (see fig. 1)
5. Add 50 µl of standard 1, 0 nMol/L to the first well. (see fig. 2)
6. Add 50 µl of standard 2, 50 nMol/L to the second well.
7. Add 50 µl of standard 3, 100 nMol/L to the third well.
8. Add 50 µl of standard 4, 250 nMol/L to the fourth well.
9. Add 50 µl of sample (serum/ plasma) to the remaining wells.
10. Add 60 µl of Biotin conjugate to each well. (see fig. 3)
11. Mix the reagents gently (see "**Preparations**").

12. Incubate 60 minutes at room temperature
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. Dilute the concentrated Streptavidin conjugate 1:100 in conjugate buffer in a clean tube or vial(10 µl to 1ml)
15. Add 100 µl of the diluted Streptavidin conjugate to each well and incubate 25 minutes. (see fig. 5)
16. Wash as in 13
17. Add 60 µl of substrate A to each well. (see fig. 6)
18. Add 60 µl of substrate B to each well.
19. Mix the reagents gently (see "**preparations**").
20. Incubate for 9-10 minutes in the dark (e.g. cover the wells with a sheet of paper)
21. Mix the reagents gently (see "**preparations**").
22. Program the analyser on analysis number: 59
23. Read the results by eye or using a spectrophotometer.

8 INTERPRETATION OF RESULTS

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

The T4 concentration in the samples can be determined by relating them to the standards.
The degree of colour development is proportional to the T4 concentration.

Color	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 too 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.

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.

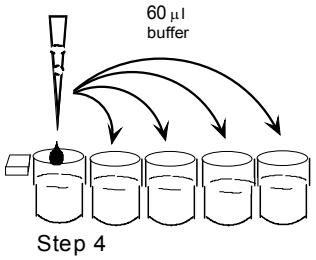

Fig. 1 Buffer procedure

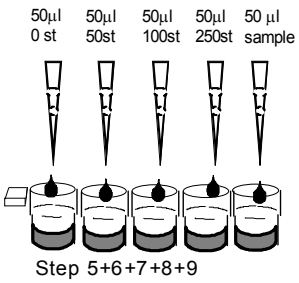

Fig. 2 Sampling procedure

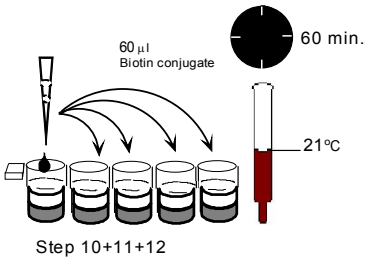

Fig. 3 Biotin Conjugate procedure

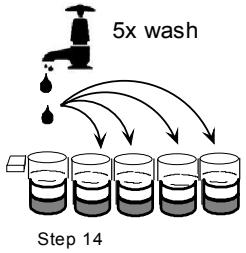

Fig. 4 Wash procedure

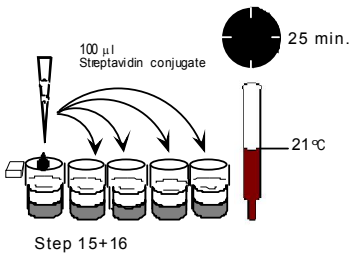

Fig. 5 Streptavidin Conjugate procedure

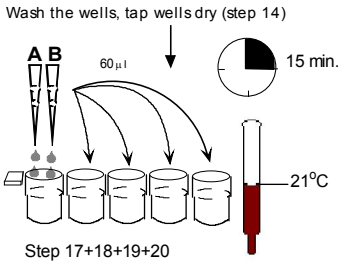

Fig. 6 Substrate procedure

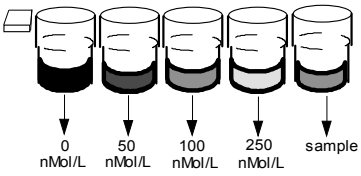

Fig. 7 Example result

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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 these products.

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