

DRG® T4 (Canine) Rapid ELISA (EIA-3441)**Revised 22 MAY 2009 (VERS. 3.0)****FOR VETERINARY USE ONLY****1 INTRODUCTION****For Veterinary use only!**

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

Up to 20% of normal dogs have decreased serum/plasma T4 total levels (Muller et al '83).

T4 total levels decrease during ageing and certain breeds (C. Spaniel, Labrador and 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 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 10-200µl (EVL)

Pipette tips (EVL)

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.

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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

- o 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.
- o 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.
- o 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.
- o 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 μ l 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 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

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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 the analyser.

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	0 nMol/L	T4 is extremely low
Dark blue-blue	<50 nMol/L	T4 is low
blue- light blue	50- 100 nMol/L	T4 is normal
Light blue- clear blue	100- 250 nMol/L	T4 is to 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.

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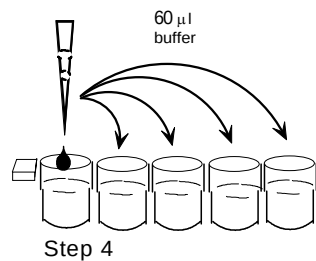


Fig. 1 Buffer procedure

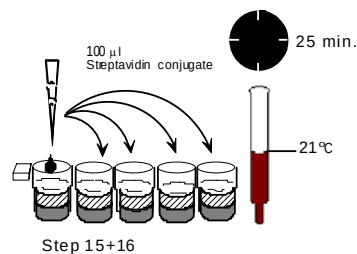


Fig. 5 Streptavidin Conjugate procedure

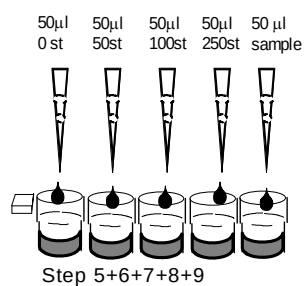


Fig. 2 Sampling procedure

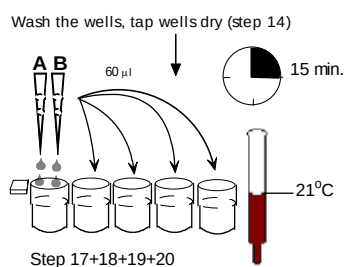


Fig. 6 Substrate procedure

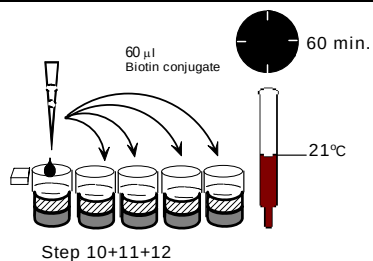


Fig. 3 Biotin Conjugate procedure

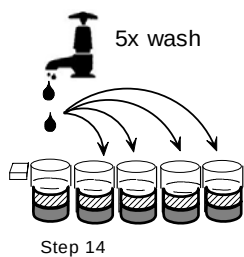


Fig. 4 Wash procedure

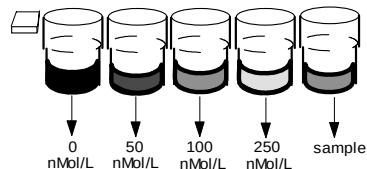


Fig. 7 Example result

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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.

SYMBOLS USED WITH DRG 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	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
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
					
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Distributed by					
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ.	