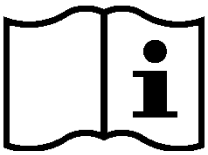
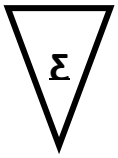


Useŕs Manual

Canine total T4 SRE

An SRE test to measure the total T4 concentration in serum and plasma samples of canine species

REF D3210-HR01



32

Nov 2021

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

1. Table of Contents

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands

Tel: +31 (0)348-412549
Web: www.evlonline.org
@: info@evlonline.eu

2. Introduction

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

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

3. Intended use of the test kit

The canine total T4 SRE is designed to detect total T4 in individual serum and plasma samples. For this purpose monoclonal anti-T4 antibodies attached to the plate will catch the 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 plate will be washed to remove unbound thyroxin. Peroxidase marked streptavidine conjugate will be added to the SRE wells. After incubation the SRE plate will be washed to remove unbound streptavidine. Substrate will be added to the SRE wells and the colour development is inversely correlated with the quantity of T4 (thyroxine) in the sample.

4. 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 32-well microtiter strip plate.
The canine serum/plasma is added together with the biotin marked T4 to the wells of the coated plate.
Colour reaction in the wells is inversely related to the concentration of thyroxin in the serum/plasma sample.

5. Contents

- 4x 8 Microtiter strips coated with monoclonal anti-T4 total antibodies
- 1x Standard 1, 0 nMol/L (green cap)
- 1x Standard 2, 50 nMol/L (brown cap)
- 1s Standard 3, 100 nMol/L (red cap)
- 1x Standard 4, 250 nMol/L (yellow cap)
- 1x streptavidin conjugate (black bottle + red cap)
- 1x Buffer (white bottle + green cap)
- 1x Biotin conjugate (white bottle + black cap)
- 1x Streptavidin conjugate buffer (black bottle + red cap)
- 1x Substrate A (white bottle + white cap)
- 1x Substrate B (black bottle + blue cap)

Supplies needed (not included)

- Precision pipette 10-200µl (EVL)
- Pipette tips (EVL)
- ELISA plate reader (the results can bet interpreted by eye, but for a more accurate and objective reading the use of the ELISA plate reader is strongly recommended)

6. Handling and storage of specimens

- The kit should be stored at 4°C.
- An open strip packet should be used within 28 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.

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 solutions.
- 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. Before starting this test read **"preparations"**.
2. Open the packet of strips and take out the amount of wells needed from the test strip, 1 for each sample and 4 extra wells for the controls. Cover the remaining strips with a part of the provided seal and store them at 4°C and use them within 10 days.
3. Use the Precision pipette 10-200µl and use a clean pipette tip **before** pipetting the buffer, standards, samples, conjugate and substrate.
4. Before testing make sure all reagents are at room temperature.

5. 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

6. Add 60µl of buffer to each well.
7. Add 60µl of standard 1, 0 nMol/L to the first well.
8. Add 60µl of standard 2, 50 nMol/L to the second well.
9. Add 60µl of standard 3, 100 nMol/L to the third well.
10. Add 60µl of standard 4, 250 nMol/L to the fourth well.
11. Add 60µl of sample (serum/plasma) to the remaining wells.
12. Add 50µl of Biotin conjugate to each well.
13. Mix the reagents gently (see **"preparations"**).
14. Incubate 60 minutes at room temperature (±21°C).
15. Turn on the analyser (when available).

16. 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

17. Add 100µl of Streptavidin conjugate to each well and incubate 25 minutes at room temperature (±21°C).

18. 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

19. Add 60µl of substrate A to each well (fig.6).
20. Add 60µl of substrate B to each well.
21. Mix the reagents gently (see **"preparations**").
22. Incubate for 7-10 minutes in the dark (e.g. cover the wells with a sheet of paper).

23. Read the absorbency values immediately (**within 10 min!**) at 620 nm on the analyser or by eye.
    Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands

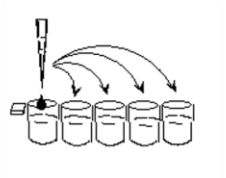
Tel: +31 (0)348-412549
Web: www.evlonline.org
@: info@evlonline.eu

9. Illustrated Test protocol

STEP: 5

- Wash procedure
- Repeat 5 times

STEP: 6

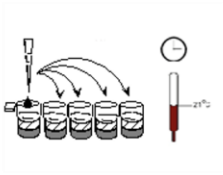


- Add 60µl of buffer to all the wells.

STEP: 7 t/m 11

- Add 60µl of standard 1, 0 nMol/L to the first well.
- Add 60µl of standard 2, 50 nMol/L to the second well.
- Add 60µl of standard 3, 100 nMol/L to the third well.
- Add 60µl of standard 4, 250 nMol/L to the fourth well.
- Add 60µl of sample (serum/plasma) to the remaining wells.

STEP: 12 t/m 14



- Add 50µl of Biotin conjugate to each well.
- Mix the reagents gently (see “preparations”).
- Incubate for 60 minutes at ±21°C.

STEP: 16

- Wash procedure
- Repeat 5 times

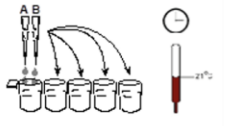
STEP: 17

- Add 100µl of Streptavidin conjugate to each well.
- Incubate for 25 minutes at ±21°C.

STEP: 18

- Wash procedure
- Repeat 5 times

STEP: 19 t/m 23



- Add 60µl of substrate A to each well.
- Add 60µl of substrate B to each well.
- Mix the reagents gently (see “preparations”)
- Incubate for 7-10 minutes in the dark at ±21°C.

Read the absorbency values immediately (within 10 min!) at 620 nm on the analyser or by eye
Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands

Tel: +31 (0)348-412549
Web: www.evlonline.org
@: info@evlonline.eu

10. Precautions

- Handle all biological material as through 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 optimal cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11. Interpretation of the test results

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

Colour	**T4 level**	**Results**
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: 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 results and available clinical information.

12. Symbols used with EVL ASSAYS

Symbol	Description
	Consult instructions for use
	European Conformity
	In vitro diagnostic device
	For research use only
	Catalogue number
	Lot/ No. / Batch code
	Contains sufficient for <n> tests
	Storage Temperature
	Expiration Date
	Legal Manufacturer
Distributed by	Distributor
Content	Content
Volume/No.	Volume / No.

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.

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands

Tel: +31 (0)348-412549
Web: www.evlonline.org
@: info@evlonline.eu