Thyroid Stimulating Hormone (TSH) Canine ELISA

For the quantitative measurement of canine TSH in serum and EDTA plasma.

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 55-TSHCA-E01

Size: 96 Wells

Version: 6-12/23-ALPCO 3.0

1 INTRODUCTION

1.1 INTENDED USE

The TSH Canine ELISA is an enzyme immunoassay for the quantitative measurement of canine TSH (thyroid stimulating hormone, thyrotropin) in serum and EDTA-plasma. For Research Use Only. Not for use in diagnostic procedures.

1.2 SUMMARY AND EXPLANATION

Thyroid stimulating hormone (TSH, thyrotropin) in dogs is similar in function to TSH found in other mammalian species, including humans. It is a glycoprotein produced by the anterior pituitary gland. Through its action on the thyroid gland, it plays a major role in maintaining normal circulating levels of the iodothyronines, T4 and T3. The production and secretion of TSH is controlled by negative feedback from circulating T4 and T3, and by the hypothalamic hormone TRH (thyrotropin releasing hormone). The TSH molecule is composed of two nonidentical subunits, α and β, that are bound together in a noncovalent manner. Within a species, the TSH α-subunit is structurally identical to the α-subunits of the related glycoprotein hormones (LH, FSH and chorionic gonadotropin). The β-subunit of TSH and the β-subunits of the related hormones are structurally hormone-specific and confer upon them their unique biological activities.

Hypothyroidism is considered a common endocrine disorder in dogs, whereas hyperthyroidism in this species is nearly unknown. Research indicates that most cases of canine hypothyroidism are primary in nature, involving impaired production of the thyroid hormones T4 and T3. In this condition, elevated TSH levels are expected. Research indicates that secondary or tertiary hypothyroidism, where thyroid hormone production is low because of hypothalamic or pituitary disease, is believed to account for less than 5% of canine hypothyroidism cases. In the latter conditions research suggests that lowered levels of TSH would be expected. Studies show that suppressed thyroid hormone levels are nonspecific indicators of the disease, since they are often observed in non-thyroid illnesses.

2 PRINCIPLE OF THE ASSAY

The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of canine TSH. The microplate is coated with anti-TSH IgG.

Calibrators and samples are pipetted into the antibody coated microplate, followed by addition of incubation buffer. Afterwards, a horseradish peroxidase-labeled antibody is added. During a two hour incubation, sandwich complexes consisting of the two antibodies and the canine TSH are formed. Non-reactive components are removed by a washing step.

A chromogenic substrate, TMB (3,3',5,5'-tetramethylbenzidine), is added to all wells. During a 30 minute incubation, the substrate is converted to a colored end product (blue) by the bound enzyme. The enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution (color changes from blue to yellow). The color intensity is directly proportional to the concentration of canine TSH present in the sample. The optical density of the color solution is measured with a microplate reader at 450 nm.

3 WARNINGS AND PRECAUTIONS

1. For Research Use Only.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing of infectious agents.
3. Blood products used in the manufacturing of this product were tested by FDA approved methods for the presence of antibody to HIV 1/2 and HIV NAT, antibody to HCV, as well as for the Hepatitis B Surface Antigen (HBsAG), and found to be negative. In addition, each blood product was tested for syphilis and found to be negative.
4. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
5. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
7. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
8. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
9. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
10. All reagents should be at room temperature (18-25°C) before use. Temperature will affect the absorbance readings of the assay.
11. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
12. Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
13. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
14. Handling should be done in accordance with the procedures defined by appropriate national and local biohazard safety guidelines or regulations.
15. Do not use reagents beyond expiry date as shown on the kit labels.
16. All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
17. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may yield slightly different results.
18. Avoid contact with Stop Solution. It may cause skin irritation and burns.
19. Some reagents contain Proclin 300, CMIT, and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
20. Chemicals and prepared or used reagents must be treated as hazardous waste according to national biohazard safety guidelines or regulations.
21. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available on ALCO's website or upon request.
22. If product information, including labeling, is incorrect or inaccurate, please contact ALPCO.

4 REAGENTS

4.1 REAGENTS PROVIDED

1. **Microtiter plate**, 12 x 8 (break apart) strips with 96 wells, ready to use; wells coated with an anti- TSH IgG antibody.

2. **Calibrators 0 - 5**, 6 vials**,** lyophilized, reconstitution required; TSH in serum matrix. The concentrations are 0, 0.2, 0.46, 1.05, 2.2 and 5.2 ng/mL**. For reconstitution see "Reagent preparation".**

3. **Enzyme Conjugate**, 1 vial, 11 mL, red, ready to use; contains a horseradish peroxidase-labeled monoclonal anti-TSH IgG antibody in a phosphate-buffered matrix.

4. **Incubation Buffer**, 1 vial, 6 mL, yellow, ready to use; phosphate-buffered matrix

5. **TMB-Substrate Solution**, 1 vial, 22 mL, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

6. **Stop Solution**, 1 vial, 7 mL, ready to use; contains 2 N Hydrochloric Acid solution. Avoid contact with Stop Solution. It may cause skin irritation and burns.

7. **Wash Buffer Concentrate**, 1 vial, 50 mL (10X concentrated); **see "Reagent preparation".**

4.2 MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable for endpoint measurements at 450 nm
- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Vortex mixer
- Microplate mixer operating at 900 rpm
- Manual or automatic equipment for microtiter plate washing
- Deioinized water
- Plastic containers for storage of the wash solution
- Semilogarithmic graph paper or software for data reduction
- Timer

4.3 REAGENT PREPARATION

Wash Buffer:
Dilute 50 mL of 10X concentrated wash solution with 450 mL deionized water to a final volume of 500 mL. The diluted Wash Buffer is stable for at least 12 weeks at room temperature (18-25˚C). Precipitates may form when stored at 2-8˚C, which should dissolve again by swirling at room temperature (18-25˚C). The wash solution should only be used when the precipitates have completely dissolved.

Calibrators:
Reconstitute lyophilized Calibrators 0 through 5 with **1.0 mL distilled water** 30 minutes before use.

4.4 STORAGE CONDITIONS

When stored at 2-8°C unopened reagents will be stable until the expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. After first opening, the reagents are stable for 30 days if used and stored properly. Microtiter wells must be stored at 2-8°C. Take care that the foil bag is sealed tightly.

Store Calibrators refrigerated; they are stable at 2-8°C for up to 7 days after reconstitution. For longer storage (up to 30 days) freeze at ≤ -20°C.

Protect TMB-Substrate Solution from light.

4.5 DISPOSAL OF THE KIT

The disposal of the kit must be done according to local and national regulations. Special information for this product is given in the Safety Data Sheet available on ALPCO's website.

4.6 DAMAGED KITS

In case of any severe damage of the kit or components, ALPCO must be informed in writing, no later than one week after receiving the kit. Severely damaged single components should not be used for an assay run. They must be stored properly pending a recommendation from ALPCO.

5 SAMPLE COLLECTION AND PREPARATION

For determination of canine TSH, serum and EDTA plasma are the preferred sample matrices. The procedure calls for 100 µL of sample per well. The samples may be stored refrigerated at 2 - 8°C for 1 week, or up to 2 months at –20°C. To avoid repeated thawing and freezing, the samples should be aliquoted.

Samples expected to contain canine TSH concentrations higher than the highest calibrator (calibrator 5) should be diluted in the Canine TSH Zero Calibrator before assay. The additional dilution step must be considered in the calculation of the results.

6 ASSAY PROCEDURE

6.1 GENERAL REMARKS

- All reagents and samples must be allowed to come to room temperature (18-25°C) before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control, or sample to avoid cross-contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

- As a general rule, the enzymatic reaction is linearly proportional to time and temperature.
- Adhere to the incubation times as stated in the instructions for use.
- Duplicate determination of calibrators, controls, and samples is recommended to identify potential pipetting errors.
- Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multi-stepper or an automatic microplate washing system. Do not allow wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Aspirate and fill all wells with care. While rinsing, check that all wells are filled precisely with 1X Working Wash Solution and that there is no residue in the wells.
- A calibrator curve must be established for every run.

7.2 ASSAY PROCEDURE

1. Prepare enough microplate wells to accommodate calibrators (0 through 5) and samples in duplicate.
2. Pipet 100 µL of each calibrator and sample using new disposable tips into the assigned wells of the microplate.
3. Dispense **50 µL** of **Incubation Buffer** into each well.
4. Add **100 µL** of **Enzyme Conjugate** to all wells.
5. Rotate for **2 hours** at room temperature (18-25˚C) on a plate mixer (900 rpm).
6. Discard the contents of the wells and wash **4 times** with **300 µL 1X working wash solution**. Remove as much wash solution as possible by tapping the microplate carefully.
7. Add **200 µL** of **TMB-Substrate Solution** to all wells.
8. Incubate without shaking at room temperature (18-25˚C) for **30 minutes** in the dark.
9. Add **50 µL** of **Stop Solution** to each well.
10. Determine the optical density of each well at **450 nm**. It is recommended to read the wells within 15 minutes.

7.3 CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard (y-axis, linear) against its corresponding concentration (X-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4-PL (4-Parameter Logistics) curve fit. A 4-Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard must be further diluted. For the calculation of the concentrations this dilution factor must be considered.

8. PERFORMANCE CHARACTERISTICS

8.1 EXAMPLE OF TYPICAL CALIBRATOR CURVE

The figure below shows typical results for TSH canine test kits. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrator	Concentration (ng/mL)	OD (450nm)
0	0	0.102
1	0.2	0.259
2	0.46	0.456
3	1.05	0.842
4	2.2	1.566
5	5.2	2.911

8.2 EXPECTED VALUES

Blood was collected from 20 apparently healthy untreated dogs (beagles) and assayed according to protocol.

		ng/mL			
Population	n	Range	Mean	P2.5	P97.5
Serum (beagles)	20	0.01 – 0.34	0.09	0.01	0.26

Laboratories should consider reference range limits as *guidelines only*. Because of differences which may exist between laboratories and locales with respect to breed, laboratory technique, and selection of reference groups, it is important for each laboratory to establish by similar means the appropriateness of adopting the reference range suggested here.

8.3 ANALYTICAL SENSITIVITY

The analytical sensitivity of the TSH Canine ELISA was calculated by adding two standard deviations from the mean of twenty-two (22) replicate analyses of *Calibrator 0*. The analytical sensitivity of the assay is 0.049 ng/mL.

8.4 REPRODUCIBILITY

8.4.1 INTRA-ASSAY

The intra-assay variation was determined by 20 replicate measurements of 3 serum samples within one run using the TSH Canine ELISA. The intra-assay variability is shown below:

	Sample 1	**Sample 2**	**Sample 3**
Mean (ng/mL)	0.26	1.33	2.27
SD (ng/mL)	0.02	0.05	0.11
CV (%)	7.2	3.9	4.7
n	20	20	20

8.4.2 INTER-ASSAY

The inter-assay variation was determined by duplicate measurements of 3 serum samples in 10 different runs using the TSH Canine ELISA. The inter-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	0.25	1.41	2.66
SD (ng/mL)	0.02	0.08	0.21
CV (%)	7.7	6.0	7.8
n	10	10	10

8.5 LINEARITY

In dilution experiments sera with high TSH concentrations were diluted with the zero calibrator and assayed in the Canine TSH canine kit.

Sample	Dilution Factor	Measured Concentration	Expected Concentration	Recovery
		[ng/mL]	[ng/mL]	[%]
1	-	4.21	-	-
	1:2	1.88	2.10	90
	1:4	1.03	1.05	98
	1:8	0.54	0.53	103
2	-	4.29	-	-
	1:2	2.01	2.15	93
	1:4	0.93	1.07	86
	1:8	0.48	0.54	89
3	-	1.16	-	-
	1:2	0.72	0.58	125
	1:4	0.35	0.29	122
	1:8	0.17	0.14	114

9. LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instructions and with adherence to GLP (Good Laboratory Practice). Any improper handling of samples or modification of this test might influence the results.

9.1 INTERFERING SUBSTANCES

- Do not use any hemolytic, ecteric, or lipemic samples to avoid inteferences.
- Samples containing sodium azide should not be used in the assay.
- Non-specific interferences with this in vitro immunoassay cannot be excluded. If implausible results are suspected, they should be considered invalid and verified by further testing.

9.2 DRUG INTERFERENCES

Currently no substances (drugs) are known to us which have an influence on the measurement of TSH in a sample. Any medication should be considered when assessing results.

10. LEGAL ASPECTS

10.1 RELIABILITY OF RESULTS

The assay must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include a sufficient number of controls within the test procedure for validating the accuracy and precision of the test. The assay results are only valid if all controls meet the specified ranges and all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ALPCO.

10.2 LIABILITY

Any modification of the kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall assay. Such modification and/or exchanges invalidate any claim for replacement.

Regardless, in the event of any claim, ALPCO's liability is not to exceed the value of the kit. Any damage caused to the test kit during transportation is not subject to the liability of ALPCO.

11. REFERENCES

1. Ruschig, S., Kraft, W. Bestimmung von caninem Thyroidea-stimulierendem Hormon (cTSH) im Blutserum des Hundes und seine Reaktion im TRH-Stimulationstest. Tierärztl Prax 1996; 24: 479-483.
2. Iversen, L., Hoier, R., Jensen, A.L. , Skydsgaard, M., Koch, J. Evaluation of the analytical performance on an enzyme immunometric assay (EIA) designed to measure endogenous thyroid-stimulating hormone (TSH) in canine serum samples. J. Vet. Med. A 45 (1998): 93-98.
3. Ramsey, I.K., Evans, H., Herritage, M.E. Thyroid-stimulating hormone and total thyroxine concentrations in euthyroid, sick euthyroid and hypothyroid dogs. Small Animal Practice 38 (1997): 540-545.
4. Cortese, L., Oliva, G., Verstegen, J., Ciaramella, P., Persechino, A. Hyperprolactinaemia and galactorrhoea associated with primary hypothyroidism in a bitch. Small Animal Practice 38 (1997): 572-575.

12. SHORT INSTRUCTION

(all sample sizes given in µL)

MP Well		CAL 0	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	Sample
	ng/mL	0	0.20	0.46	1.05	2.20	5.20	
Steps	**Solution**							
Pipet	Calibrator	**100**	**100**	**100**	**100**	**100**	**100**	**-**
Pipet	Sample	**-**	**-**	**-**	**-**	**-**	**-**	**100**
Pipet	Incubation Buffer	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Pipet	Enzyme Conjugate	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Incubate for **2h** at RT (18-25˚C) on a shaker (900 rpm)								
Decant Wash **4x with 300 µL** of 1X working wash solution								
Pipet	Substrate Solution	**200**	**200**	**200**	**200**	**200**	**200**	**200**
Incubate for **30 min** at RT (18-25˚C)**in the dark**								
Pipet	Stop Solution	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Read at **λ = 450 nm**								