



## User's Manual

# Canine TSH ELISA Kit



DEIASL210



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

The TSH canine ELISA is an enzyme immunoassay for the quantitative measurement of canine TSH (thyrotropin) in serum and EDTA-plasma.

### General Description

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,  $\alpha$  and  $\beta$ , that are bound together in a noncovalent manner. Within a species, the TSH  $\alpha$  subunit is structurally identical to the  $\alpha$  subunits of the related glycoprotein hormones (LH, FSH and chorionic gonadotropin). The  $\beta$  subunit of TSH and the subunits of the related hormones are structurally hormonespecific, and account for their unique biological activities.

Hypothyroidism is considered to be a common endocrine disorder in dogs, whereas hyperthyroidism in this species is nearly unknown. Dogs mostly suffer from primary hypothyroidism, involving impaired production of the thyroid hormones, T4 and T3. In this condition, elevated TSH levels are expected. Secondary or tertiary hypothyroidism, where thyroid hormone production is low as a consequence of hypothalamic or pituitary disease, is believed to account for less than 5% of canine hypothyroidism cases. In the latter conditions, decreased levels of TSH would be expected. Usually, hypothyroidism in dogs is suspected on the basis of clinical history and the presence of decreased levels of thyroid hormones. However, suppressed thyroid hormone levels are nonspecific indicators of the disease, since they are often observed in nonthyroid illnesses. The evaluation of thyroid function and the diagnosis of hypothyroidism in dogs can be greatly improved through the use of the valid assay for the determination of canine TSH.

### Principles of Testing

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 an antibody specific for canine TSH. Standards and samples are pipetted into the antibody coated microplate. Afterwards, a horseradish peroxidase labeled antibody is added. During a two hour incubation sandwich complexes consisting of the two antibodies and the canine TSH is formed. Non-reactive components are removed by a washing step. A chromogenic substrate, TMB (3,3',5,5'-Tetra-Methyl-Benzidine), is added to all wells. During a 30 minutes incubation, the substrate is converted to a colored end product (blue) by the bound enzyme. Enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution (change from blue to yellow). The color intensity is direct 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.

### Reagents And Materials Provided

1. Microtiter Plate: 12x8 (break apart) strips with 96 wells; Wells coated with an anti-canine TSH antibody.

Ready to use.

2. Standards: highly purified canine TSH in serum. Lyophilized, reconstitution required\*

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Component	Concentration	Volume/Vial
Standard A	0 ng/ml	1 ml
Standard B	0.2 ng/ml	1 ml
Standard C	0.46 ng/ml	1 ml
Standard D	1.05 ng/ml	1 ml
Standard E	2.2 ng/ml	1 ml
Standard F	5.2 ng/ml	1 ml

3. Enzyme Conjugate: contains a horseradish peroxidase-labeled polyclonal anti canine TSH antibody, in a buffered matrix, 1 x 11 ml, red. Ready to use.
4. TMB Substrate Solution: contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix, 1 x 22 ml. Ready to use.
5. Stop Solution: contains 2 N Hydrochloric Acid solution, 1 x 7 ml. Ready to use.
6. Wash Solution Concentrate (10x): 1 x 50 ml

## Materials Required But Not Supplied

1. Microplate reader capable for endpoint measurements at 450 nm
2. Vortex mixer
3. Microplate mixer operating more than 600 rpm
4. Distilled water
5. Graduated cylinders for 500 ml
6. Plastic containers for storage of the wash solution
7. Adjustable pipette for up to 1000 µl
8. Dispenser or repeatable pipet for 100 µl and 200 µl

## Storage

When stored at 2°C to 8°C unopened reagents will be stable until 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 Standards refrigerated, they will be stable at 2-8 °C for 7 days after reconstitution. For longer storage aliquot and freeze at -20 °C.

Protect TMB-Substrate Solution from light.

## Specimen Collection And Preparation

For determination of canine TSH serum and EDTA-plasma are the preferred sample matrices. The procedure calls for 100 µl sample per well. The samples may be stored refrigerated at 2 - 8 °C for one week, or up to two 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 Standard F should be diluted in the

Canine TSH Standard A before assay.

The additional dilution step has to be taken into account for the calculation of the results.

## Reagent Preparation

Wash Solution:

Dilute with 450 ml dist. water to a final volume of 500 ml.

The diluted Wash Solution is stable for 12 weeks at room temperature.

Standards:

Reconstitute lyophilized Standard A through F with 1.0 ml dist. water 30 minutes before use.

## Assay Procedure

Before Assay Notes:

1. All reagents and samples must be allowed to come to room temperature (18-25°C) before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. 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.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Respect the incubation times as stated in this instructions for use.
7. Duplicate determination of standards, controls and samples is recommended in order to identify potential pipetting errors.
8. For internal quality control we suggest to use Canine Control. For more information please contact the manufacturer.

Assay Steps

1. Prepare a sufficient number of microplate wells to accommodate Standards (A through F) and patient samples in duplicates.
2. Pipet 100 µl of each Standard, Control and sample into the wells prepared.
3. Add 100 µl of Enzyme Conjugate to all well.
4. Rotate for 2 hours on a plate mixer (600 – 900 rpm).
5. Discard the content of the wells and wash 4 times with 300 µl buffered wash solution. Remove as much wash solution as possible by beating the microplate carefully.
6. Add 200 µl of TMB-Substrate Solution to all wells.
7. Incubate without shaking for 30 minutes in the dark.
8. Add 50 µl of Stop Solution to each well and mix carefully.
9. Determine the absorbance of each well at 450±10 nm. It is recommended to read the wells within 15

minutes.

## Quality Control

Good laboratory practice requires that controls are run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate included in the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microtiter plate reader, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

## Calculation

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
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. 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 have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

## Typical Standard 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.

Standard	Concentration (ng/mL)	OD (450nm)
A	0	0.071
B	0.2	0.233
C	0.46	0.423
D	1.05	0.852
E	2.2	1.569
F	2.2	3.092

## Reference Values

Blood was collected from 30 apparently healthy dogs. A mean canine TSH value of 0.216 ng/ml was found, with an absolute range of 0.017 to 0.591 ng/ml.

Laboratories should consider reference range limits as guidelines only. Because of differences which may exist between laboratories and locales with respect to population, 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.

## Precision

### Intra-assay

The intra-assay variation was determined by 18 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)	0.57	0.92	2.57
SD (ng/ml)	0.04	0.09	0.28
CV (%)	7.0	9.9	11.0
n=	18	18	18

### 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)	0.53	0.97	3.15
SD (ng/ml)	0.05	0.08	0.15
CV (%)	10.2	8.5	4.8
n=	10	10	10

## 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 Standard A. The analytical sensitivity of the assay is 0.00002 ng/ml.

## Linearity

In dilution experiments sera with high antibody concentrations were diluted with the Standard A and assayed in the TSH canine kit.

Sample	Dilution Factor	measured Concentration (ng/ml)	expected Concentration (ng/ml)	Recovery %
1	-	1.81	-	-
	1:2	0.92	0.91	101
	1:4	0.45	0.45	100
	1:8	0.22	0.23	96
2	-	2.91	-	-
	1:2	1.50	1.46	103
	1:4	0.72	0.73	99
	1:8	0.37	0.36	103
3	-	3.41	-	-
	1:2	1.76	1.71	103
	1:4	0.79	0.85	93
	1:8	0.37	0.43	86

## Precautions

1. The kit is strictly intended for veterinary use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
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. 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.
4. 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.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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 result in colored solution. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedure defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. 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 vary slightly.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national

biohazard safety guideline and regulation.

19. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

## Limitations

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