LDN®
IMMUNOASSAYS AND SERVICES
BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY
LABOR DIAGNOSTIKA NORD GmbH & Co.KG | Am Eichenhain 1 | 48531 Nordhorn | Germany | Tel. +49 5921 8197-0 | Fax +49 5921 8197-222 | info@ldn.de | www.ldn.de

Instructions for use

TSH canine ELISA

REF **AR E-8500R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

TSH canine ELISA

1. INTRODUCTION

1.1 INTENDED USE

The TSH canine ELISA is an enzyme immunoassay for the quantitative measurement of canine TSH (thyrotropin) in serum and EDTA plasma. For manual processing! The usage of laboratory automats is the user's sole responsibility. The kit is intended for single use only.

1.2 DESCRIPTION OF THE ANALYTE

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 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.

2. PRINCIPLE

The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format with liquid phase incubation for the quantitative measurement of canine TSH in serum or EDTA plasma samples. The microplate is coated with anti-TSH IgG.
Standards 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 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 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 (OD) of the color solution is measured with a microplate reader at 450 nm.

3. WARNINGS AND PRECAUTIONS

1. This kit is intended for laboratory 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. Each donor unit used in the manufacturing of this product was 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 donor and/or donor unit was tested for Syphilis and found to be negative. 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 turn solution colored. 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.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.

11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex 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 procedures 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 result slightly different.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4. REAGENTS

4.1 REAGENTS PROVIDED

AR E-8531 **96** **Microtiter Plate** – Ready to use

Content: 12x8 (break apart) strips with 96 wells; wells coated with an anti-TSH antibody IgG.

Standards – Lyophilized, reconstitution required*

Cat. no.	Component	Standard	Concentration	Volume/Vial
AR E-8501	**STANDARD A**	**Standard A**	0 ng/ml	1 ml
AR E-8502	**STANDARD B**	**Standard B**	0.2 ng/ml	1 ml
AR E-8503	**STANDARD C**	**Standard C**	0.46 ng/ml	1 ml
AR E-8504	**STANDARD D**	**Standard D**	1.05 ng/ml	1 ml
AR E-8505	**STANDARD E**	**Standard E**	2.2 ng/ml	1 ml
AR E-8506	**STANDARD F**	**Standard F**	5.2 ng/ml	1 ml

Content: TSH in serum matrix.

***For reconstitution see "Reagent preparation" (4.3).**

AR E-8540 **CONJUGATE** **Enzyme Conjugate** – Ready to use

Content: Contains a horseradish peroxidase-labeled monoclonal anti-TSH IgG antibody, in a phosphate-buffered matrix.

Volume: 1 x 11 ml, red

AR E-8513 **INC-BUFF** **Incubation Buffer** – Ready to use

Content: Phosphate-buffered matrix.

Volume: 1 x 6 ml, yellow

AR E-0055 **SUBSTRATE** **TMB-Substrate Solution** – Ready to use

Content: Contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

Volume: 1 x 22 ml, clear

AR E-0080 **STOP-SOLN** **Stop Solution** – Ready to use

Content: Contains 2 N Hydrochloric acid solution.
Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 7 ml

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H335 May cause respiratory irritation.

AR E-0030 **WASH-CONC** **10x** **Wash Solution** – 10X concentrated

Volume: 1 x 50 ml

See "Reagent preparation" (4.3).

4.2 MATERIALS REQUIRED BUT NOT PROVIDED

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes and multichannel pipettes with disposable pipette tips
- Microtiter plate mixer operating at 900 rpm
- Manual or automatic equipment for microtiter plate washing
- Absorbent paper
- Deionized water
- Timer
- Semilogarithmic graph paper or software for data reduction
- Vortex mixer

4.3 REAGENT PREPARATION

Wash Solution:
Dilute 50 ml of 10x concentrated Wash Solution with 450 ml deionized water to a final volume of 500 ml. The diluted Wash Solution 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.

Standards:
Reconstitute lyophilized Standards A through F with **1.0 ml dist. water** 30 minutes before use.

4.4 STORAGE CONDITIONS

When stored at 2 – 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, after reconstitution for up to seven days at 2 – 8 °C, for longer storage up to 30 days aliquoted at ≤ -20 °C.
Protect TMB-Substrate Solution from light.

4.5 DISPOSAL OF THE KITS

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 DAMAGED TEST KITS

In case of any severe damage of the test kit or components, the manufacturer have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

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 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.

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 in order 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.
- Respect the incubation times as stated in this instructions for use.

- Duplicate determination of standards, controls and samples is recommended in order 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 multistepper, respectively, or an automatic microtiter plate washing system. Do not allow wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Solution, and that there are no residues in the wells.
- A standard curve must be established for every run.
- For internal quality control we suggest to use Canine Control. For more information please contact the manufacturer.

6.2 ASSAY PROCEDURE

1.	Prepare a sufficient number of microplate wells to accommodate standards (A through F) and samples in duplicates.
2.	Pipet **100 µl** of each **standard**, **control** and **sample** with new disposable tips into the appropriate 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 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.
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.	Stop reaction by adding **50 µl** of **Stop Solution** to each well.
10.	Determine the optical density of each well at **450 nm** and read the wells within 15 minutes.

6.3 CALCULATION OF RESULTS

1. Calculate the average optical density values for each set of standards, controls and samples.
2. The obtained optical densities of the standards (y-axis, linear) are plotted against their corresponding concentrations (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. 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 and assayed again. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 EXAMPLE OF 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	0.102
B	0.2	0.259
C	0.46	0.456
D	1.05	0.842
E	2.2	1.566
F	5.2	2.911

7. EXPECTED VALUES

Blood was collected from 20 apparently healthy untreated dogs (Beagles) and assayed after protocol.

		ng/ml				
Population	**n**	**Range**	**Mean**	**Median**	**p2.5**	**p97.5**
Serum (Beagles)	20	0.01 – 0.34	0.09	0.08	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. 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. 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 external Canine Control Set 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.
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 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.

9. PERFORMANCE CHARACTERISTICS

9.1 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 *Standard A*. The analytical sensitivity of the assay is 0.049 ng/ml.

9.2 REPRODUCIBILITY

9.2.1 INTRA-ASSAY

The intra-assay variation was determined by 20 replicate measurements of 3 serum samples within one run using the 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

9.2.2 INTER-ASSAY

The inter-assay variation was determined by duplicate measurements of 3 serum samples in 10 different runs using the 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

9.3 LINEARITY

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

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

10. LIMITATIONS OF PROCEDURE

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.

10.1 INTERFERING SUBSTANCES

- Do not use any hemolytic, icteric or lipemic specimens to avoid any interferences.
- Samples containing sodium azide should not be used in the assay.
- Non-specific interferences with this in vitro immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing. For diagnostic purposes, results should always be considered only in conjunction with the clinical picture and further diagnostic tests.

10.2 DRUG INTERFERENCES

Until today no substances (drugs) are known to us, which have an influence to the measurement of TSH in a sample. Any medication should be taken into account when assessing the results.

11. LEGAL ASPECTS

11.1 RELIABILITY OF RESULTS

The test 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 included a sufficient number of controls within the test procedure for validating the accuracy and precision of the test. The test 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 the manufacturer.

11.2 THERAPEUTIC CENSEQUENCES

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient therapeutic consequences should be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 LIABILITY

Any modification of the test 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 test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12. 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.

13. REVISION HISTORY OF INSTRUCTION FOR USE

Changes compared to the previous version 5.0 to the current version 6.0.

New, revised test version
Section 2 updated
Section 3 updated

Section 4 Incubation buffer added, adaptation of component description
Section 4.4 updated storage condition of standards
Section 6.1 addition information to the washing procedure
Section 6.2 additional step (3) (adding 50 µl incubation buffer)
Section 7 expected values updated
Section 9 new test specifications
Section 13 change history of the package insert added
Section 14 additional pipetting step with incubation buffer added
General Editorial changes

14. SHORT INSTRUCTION

(all sample sizes given in µl)

MP Well		A	B	C	D	E	F	Sample
	ng/ml	0	0.20	0.46	1.05	2.20	5.20	
Steps	**Solution**							
Pipet	Standard	**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 buffered 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**								

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!