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AccuBind®
ELISA Microwells

# Canine Thyrotropin (Canine TSH) AccuBind® ELISA Test System

***Product Code: 12325-300***

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Thyrotropin Concentration in Canine plasma or serum by a Microplate Enzyme Immunoassay, Colorimetric**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Measuring canine thyroid-stimulating hormone (TSH, or thyrotropin) by immunoassay is a key tool in evaluating thyroid function in dogs. Thyroid-stimulating hormone (TSH, thyrotropin) in dogs is a glycoprotein hormone produced by the anterior pituitary gland. It consists of two non-covalently linked subunits: a common alpha subunit shared with other glycoprotein hormones and a hormone-specific beta subunit. The canine alpha subunit comprises 96 amino acids with a calculated molecular weight of 10,693 Da and shares 73% sequence homology with the human alpha subunit. The beta subunit consists of 118 amino acids, has 91% homology with human TSH beta, and contains six intramolecular disulfide bonds. These structural features, including specific glycosylation sites, are critical for antibody recognition in immunoassays[1-6].

Canine TSH that regulates thyroid hormone (T4 and T3) production through a negative feedback loop. In primary hypothyroidism—the most common form in dogs—impaired thyroid hormone output typically leads to elevated TSH levels, while secondary or tertiary hypothyroidism (less than 5% of cases) shows low TSH. .Immunoassays for canine TSH, such as enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays, use antibodies specific to canine TSH in a sandwich format. These provide quantitative results from serum or plasma samples and are typically combined with total T4 or free T4 measurements for accurate diagnosis, as TSH alone has high specificity but lower sensitivity[1-6].

In this method, TSH calibrator, unknown specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various Canine TSH antibodies and native Canine TSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known canine thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyrotropin concentration.

## 3.0 PRINCIPLE

**Immunoenzymometric assay (TYPE 3):**
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-Canine TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + {}_{AgCanine\ TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - {}_{AgCanine\ TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$_{AgCanine\ TSH}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme -Polyclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - {}_{AgCanine\ TSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(p)} - {}_{AgCanine\ TSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ immobilized complex

$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the well surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Materials Provided:**

**A. Canine TSH Calibrators – 1ml/vial - Icons A-G**
Seven (7) vials of references for Canine TSH Antigen at levels of 0(**A**), 0.2(**B**), 0.5(**C**), 1.0(**D**), 2.0 (**E**), 4.0 (**F**) and 8.0 (**G**) in ng/ml. Store at 2-8°C. A preservative has been added.
**Note**: The calibrators, protein based, were calibrated using reference preparation, which was prepared from canine pituitaries.

**B. Canine TSH Enzyme Reagent – 12ml/vial - Icon**
One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

**C. Streptavidin Coated Plate – 96 wells – Icon**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**D. Wash Solution Concentrate – 20 ml/ml - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**E. Substrate SN – 12ml/vial - Icon $S^N$**
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

**F. Stop Solution – 8ml/vial - Icon**
One (1) vial containing a strong acid (0.5N $H_2SO_4$). Store at 2-8°C.

**G. Product Instructions.**

**Note 1:** Do not use reagents beyond the kit expiration date.

**Note 2:** Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

**Note 3:** Above reagents are for a single 96-well microplate.

**4.1 Required But Not Provided:**
1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

## 5.0 PRECAUTIONS

***For In Vitro Diagnostic Use***
***Not for Internal or External Use in Humans or Animals***
**Safe disposal of kit components must be according to local regulatory and statutory requirement.**

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type or plasma, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

**In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.**

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, (100µl) 0.100 ml of the specimen is required.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or de-ionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

***Note1: Do not use the working substrate if it looks blue.***
***Note 2: Do not use reagents that are contaminated or have bacteria growth.***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27° C).*

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the Canine TSH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature. ******
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

**DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**

9. Incubate at room temperature for twenty (20) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
2. Plot the absorbance for each duplicate serum reference versus the corresponding Canine TSH concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Canine TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.460) intersects the dose response curve at (1.2ng/ml) Canine TSH concentration (See Figure 1).

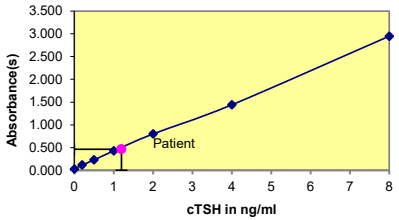
**Note:** Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**EXAMPLE 1**

| Sample I.D. | Well Number | Abs | Mean Abs | Value (µIU/ml) |
|---|---|---|---|---|
| **Cal A** | A1 | 0.026 | 0.028 | 0 |
| | B1 | 0.029 | | |
| **Cal B** | C1 | 0.110 | 0.115 | 0.2 |
| | D1 | 0.120 | | |
| **Cal C** | E1 | 0.217 | 0.228 | 0.5 |
| | F1 | 0.239 | | |
| **Cal D** | G1 | 0.421 | 0.427 | 1.0 |
| | H1 | 0.433 | | |
| **Cal E** | A2 | 0.790 | 0.799 | 2.0 |
| | B2 | 0.809 | | |
| **Cal F** | C2 | 1.470 | 1.461 | 4.0 |
| | D2 | 1.451 | | |
| **Cal G** | E2 | 2.924- | 2.949 | 8.0 |
| | F2 | 2.647 | | |
| **Control** | G2 | 0.800 | 0.460 | **1.2** |
| | H2 | 0.443 | | |

| | | | | |
|---|---|---|---|---|
| | B3 | 0.477 | | |

**Figure 1**




*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

## 11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance of calibrator G' (8ng/ml) should be ≥ 1.3.
2. If used, 2 of 3 quality control pools should be within the established range.

## 12.0 RISK ANALYSIS

*The SDS is available at https://www.monobind.com/safety-data-sheets and the Risk Analysis Form may be requested.*

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. Patient specimens with Canine TSH concentrations over 4ng/ml may be diluted (1:2) with the '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (2).
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

### 12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the Test System have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Canine TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, thyrotropin concentration alone is not sufficient to assess clinical status.**
7. Canine TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
8. A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.
9. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

## 13.0 EXPECTED RANGES OF VALUES

A large multi-breed cohort of 693 healthy dogs, the combined nonparametric reference interval for TSH was approximately <0.58 ng/mL (upper limit), with median values varying by breed from 0.07 ng/mL (e.g., Alaskan Malamute, Golden Retriever) to 0.26 ng/mL (Collie) .Journal of Veterinary Diagnostic Investigation 2015, Vol. 27(6) 716–726.27

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" animals is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

The within and between assay precisions of the Canine TSH AccuBind® Test System were determined by analyses on three different levels of pool control sera. The number (N), mean (X) value, standard deviation **(σ)** and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**
Within Assay Precision (Values in ng/ml)

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Pool 1 | 22 | 0.16 | 0.007 | 4.4% |
| Pool 2 | 22 | 1.07 | 0.032 | 3.0% |
| Pool 3 | 22 | 2.07 | 0.073 | 3.5% |

**TABLE 3**
Between Assay Precision* (Values in µIU/ml)

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Pool 1 | 10 | 0.17 | 0.016 | 9.4% |
| Pool 2 | 10 | 1.03 | 0.079 | 7.7% |
| Pool 3 | 10 | 2.03 | 0.014 | 6.9% |

### 14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

For I hr incubation = 0.05 ng/ml

## 15.0 REFERENCES


1. Boretti FS, Reusch CE. Endogenous TSH in the diagnosis of hypothyroidism in dogs. Schweiz Arch Tierheilkd. 2004;146:183-188.
2. Iversen L, et al. Evaluation of the analytical performance of an enzyme immunometric assay for canine TSH. J Vet Med A. 1998;45:93-98.
3. MSD Veterinary Manual: States that TSH remains within reference range in 20–40% of dogs with confirmed primary hypothyroidism, confirming its low sensitivity. Always interpret with T4/fT4, clinical signs, and history
4. Scott-Moncrieff JC, Nelson RW, Bruner JM, Williams DA. Comparison of serum concentrations of thyroid-stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. J Am Vet Med Assoc. 1998;212(3):387-391. (Reported 63% sensitivity and 88% specificity, improving to 100% specificity when combined with low T4.
5. Ferguson DC. Testing for hypothyroidism in dogs. Vet Clin North Am Small Anim Pract. 2007;37(4):647-669. (Detailed review of the canine TSH assay, its limitations, and interpretation strategies Beck-Peccoz P, Persani L, "Variable biological activity of thyroid stimulating hormone", *Eur J Endocrinol,* 131, 331-340 (1994).
6. Mooney CT. Canine hypothyroidism: a review of aetiology and diagnosis. N Z Vet J. 2011;59(3):105-11



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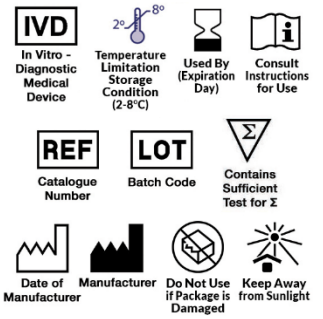
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