



IMMULITE®

Canine TSH

**For use on the IMMULITE®
and IMMULITE® 1000 systems**

DPC®

IMMULITE®/IMMULITE® 1000 Canine TSH

Intended Use

For *in vitro* veterinary use with the IMMULITE and IMMULITE 1000 Analyzers — for the quantitative measurement of canine thyroid stimulating hormone (canine thyrotropin, cTSH) in serum. It is intended strictly for *in vitro* veterinary use as an aid in the assessment of thyroid status in dogs.

Catalog Numbers: **LKKT1** (100 tests), **LKKT5** (500 tests)

Test Code: **TK9** Color: **Dark Pink**

Summary and Explanation of the Test

Thyroid stimulating hormone (TSH, thyrotropin) in dogs is similar in function and structure to TSH found in other mammalian species, including humans. TSH 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 (luteinizing hormone, follicle stimulating hormone 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 to be a common endocrine disorder in dogs, whereas hyperthyroidism in this species is relatively unknown. 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. 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, lowered levels of TSH would be expected. Usually, hypothyroidism in dogs is suspected on the basis of clinical history and the presence of lowered levels of thyroid hormones. However, suppressed thyroid hormone levels are nonspecific indicators of the disease, since they are often observed in nonthyroidal illnesses. The evaluation of thyroid function and the diagnosis of hypothyroidism in dogs can be greatly improved through the use of a valid assay for the determination of canine TSH.

Principle of the Procedure

IMMULITE/IMMULITE 1000 Canine TSH is a solid-phase, two-site chemiluminescent immunometric assay.

Incubation Cycles: 1 × 60 minutes.

Specimen Collection

The animal need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes and separate the serum from the cells.

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence the results should be interpreted with caution.

The use of an ultracentrifuge is recommended to clear lipemic samples.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants.

IMMULITE/IMMULITE 1000 Canine TSH

has not been tested with all possible variations of tube types.

Volume Required: 25 μ L serum. (Sample cup should contain at least 100 μ L more than the total volume required.)

Storage: 1 week at 2–8°C or 2 months (aliquotted) at –20°C.

Warnings and Precautions

For *in vitro* veterinary use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Canine TSH Test Units (LKT1)

Each barcode-labeled unit contains one bead coated with monoclonal murine anti-cTSH antibody. Stable at 2–8°C until expiration date.

LKKT1: 100 units. **LKKT5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

Canine TSH Reagent Wedge (LKT2)

With barcode. 7.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-cTSH antibody in buffer, with preservative. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.

LKKT1: 1 wedge. **LKKT5:** 5 wedges.

Canine TSH Adjustors (LKTL, LKTH)

Two vials (Low and High) of lyophilized canine TSH in cTSH-free canine serum/buffer matrix, with preservative. Reconstitute each vial with **4.0 mL** distilled or deionized water, and mix by *gentle* inversion. Stable at 2–8°C for 7 days after reconstitution, or for 6 months (aliquotted) at –20°C.

LKKT1: 1 set. **LKKT5:** 2 sets.

Kit Components Supplied Separately

LSUBX: Chemiluminescent Substrate

LPWS2: Probe Wash

LKPM: Probe Cleaning Kit

LCHx-y: Sample Cup Holders (barcoded)

LSCP: Sample Cups (disposable)

LSCC: Sample Cup Caps (optional)

K9CON: A bi-level, canine serum-based control, containing canine TSH as one of multiple assayed constituents.

K9TCM: A bi-level, canine serum-based control module, containing canine TSH as one of three assayed constituents.

Also Required
Sample transfer pipets; distilled or deionized water; controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE or IMMULITE 1000 Operator's Manual.

See the IMMULITE or IMMULITE 1000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Recommended Adjustment Interval:
2 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of canine TSH.

Expected Values

Based on its relation to DPC's Coat-a-Count Canine TSH assay (see the Method Comparison section), the IMMULITE Canine TSH assay can be expected to

have the following approximate reference ranges

Nondetectable to 0.5 ng/mL.

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges for the diagnostic evaluation of canine results.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise specified, all results were generated on canine samples collected in tubes without anticoagulants, gel barriers, or clot-promoting additives.)

Calibration Range: Up to 12 ng/mL

Analytical Sensitivity: 0.01 ng/mL

High-dose Hook Effect:
None up to 5,000 ng/mL.

Intraassay Precision (Within-Run):
Statistics were calculated for samples from the results of 20 replicates in a single run. (See "Intraassay Precision" table.)

Interassay Precision (Run-to-Run):
Statistics were calculated for samples assayed in 10 different runs. (See "Interassay Precision" table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three TSH solutions (4.3, 8.5 and 18 ng/mL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibodies used in the IMMULITE Canine TSH procedure are highly specific for canine TSH, with negligible crossreactivity to related canine pituitary glycoprotein hormones such as FSH, HCG and LH.

Hemolysis: Presence of packed red blood cells in concentrations up to 25 μ L/mL has no effect on results, within the precision of the assay.

Lipemia: Presence of triglycerides in concentrations up to 5,000 mg/dL has no effect on results, within the precision of the assay.

Method Comparison: The assay was compared to DPC's Coat-A-Count Canine TSH IRMA on 61 canine samples.

(Concentration range: approximately 0.04 to 9.0 ng/mL. See graph.) By linear regression:

(IML) = 1.00 (CAC IRMA) + 0.02 ng/mL
r = 0.996

Means:
1.39 ng/mL (IMMULITE)
1.37 ng/mL (CAC IRMA)

References

- 1) Ferguson DC. Thyroid function tests in the dog. *Veterinary Clinics of North America: Small Animal Practice* 1984;14:783-808.
- 2) Panciera DL. Hypothyroidism in dogs: 66 cases (1987-1992). *JAVMA* 1994;204:761-7.
- 3) Nesbitt GH, Izzo J, Peterson L, Wilkins RJ. Canine hypothyroidism: A retrospective study of 108 cases. *JAVMA* 1980;177:1117-22.
- 4) Nachreiner RF, Refsal KR. Radioimmunoassay monitoring of thyroid hormone concentration in dogs on thyroid replacement therapy: 2,674 cases (1985-1987). *JAVMA* 1992;201:623-9.
- 5) Budsberg SC, Moore GE, Klappenbach K. Thyroxine-responsive unilateral forelimb lameness and generalized neuromuscular disease in four hypothyroid dogs. *JAVMA* 1993;202:1859-60.
- 6) Beale KM. Current diagnostic techniques for evaluating thyroid function in the dog. *Vet Clin North Am* 1990;20:1429-41.
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- 8) Gonzalez E, Quadri SK. Effects of aging on the pituitary-thyroid axis in the dog. *Exp Gerontol* 1988;23:151-60.
- 9) Kaptein EM, Hays MT, Ferguson DC. Thyroid hormone metabolism: a comparative evaluation. *Vet Clin North Am* 1994;24:431-63.
- 10) Kempainen RJ, Clark TP. Etiopathogenesis of canine hypothyroidism. *Vet Clin North Am* 1994;24:467-76.
- 11) Scarlett JM. Epidemiology of thyroid diseases of dogs and cats. *Vet Clin North Am* 1994;24:477-86.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 13485:2003.

Tables and Graphs

Intraassay Precision (ng/mL)

	Mean	SD	CV
1	0.20	0.01	5.0%
2	0.50	0.02	4.0%
3	1.6	0.05	3.1%
4	2.6	0.10	3.8%

Interassay Precision (ng/mL)

	Mean	SD	CV
1	0.16	0.01	6.3%
2	0.27	0.02	7.4%
3	2.8	0.23	8.2%

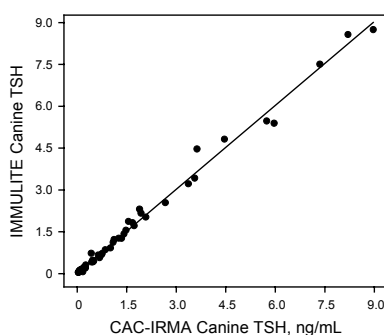
Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	16 in 16	5.1	—	—
	8 in 16	2.3	2.6	93%
	4 in 16	1.2	1.3	93%
	2 in 16	0.6	0.6	97%
	1 in 16	0.3	0.3	94%
2	16 in 16	5.96	—	—
	8 in 16	2.9	3.0	97%
	4 in 16	1.5	1.5	101%
	2 in 16	0.8	0.8	102%
	1 in 16	0.4	0.4	102%
3	16 in 16	8.12	—	—
	8 in 16	3.6	4.1	88%
	4 in 16	1.8	2.0	89%
	2 in 16	0.9	1.0	94%
	1 in 16	0.5	0.5	95%

Recovery (ng/mL)

	Solution	Observed	Expected	%O/E
1	—	0.46	—	—
	A	0.64	0.65	98%
	B	0.75	0.86	87%
	C	1.1	1.3	85%
2	—	2.8	—	—
	A	2.8	2.9	97%
	B	3.0	3.1	97%
	C	3.4	3.6	94%
3	—	5.2	—	—
	A	5.4	5.2	104%
	B	5.5	5.4	102%
	C	5.6	5.8	97%

Method Comparison



$$(IML) = 1.00 (CAC IRMA) + 0.02 \text{ ng/mL}$$

$$r = 0.996$$

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