COAT-A-COUNT®

Canine TSH IRMA

DPC®

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Intended Use

Coat-A-Count Canine TSH IRMA is an immunoradiometric assay designed 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: **IK9T1** (100 tubes).

The 100-tube kit contains less than 20 microcuries (740 kilobecquerels) of radioactive ^{125}I-polyclonal anti-cTSH.

Summary and Explanation

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.

Accordingly, DPC has developed a coated-tube immunoradiometric assay for canine TSH that can measure circulating levels of this hormone accurately and reliably with a procedure involving minimum benchwork.

Principle of the Procedure

Coat-A-Count Canine TSH IRMA is a solid-phase immunoradiometric assay based on monoclonal and polyclonal anti-cTSH antibodies: one ^{125}I-labeled anti-cTSH polyclonal antibody in liquid phase, and monoclonal anti-cTSH antibodies immobilized to the wall of a polystyrene tube. In the procedure:

Canine TSH is captured between monoclonal anti-cTSH antibodies immobilized on the inside surface of the polystyrene tube and the radiolabeled polyclonal anti-cTSH tracer.

Unbound ^{125}I-labeled anti-cTSH antibody is removed by decanting the reaction mixture and washing the tube; this reduces nonspecific binding to a very low level, and ensures excellent low-end precision.

The canine TSH concentration is directly proportional to the radioactivity present in the tube after the wash step. The radioactivity is counted using a gamma counter, after which the concentration of cTSH in the canine sample is obtained by comparing the sample counts-per-minute with those obtained for the set of calibrators provided.

Reagents to Pipet: 1

Total Incubation Time: 3 hours.

Total Counts at Iodination: approximately 300,000 cpm.

Warnings and Precautions

For *in vitro* veterinary use only.

Reagents: Store at 2–8°C in a refrigerator designated for incoming radioactive materials. Dispose of in accordance with applicable laws.

Do not use reagents beyond their expiration dates.

Some components supplied in this kit may contain human source material and/or other potentially hazardous ingredients which necessitate certain precautions:

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

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.

Water: Use distilled or deionized water.

Radioactivity
A copy of any radioisotope license certificate (Specific or General) issued to a US customer must be on file with Diagnostic Products Corporation before kits or components containing radioactive material can be shipped. These radioactive materials may be acquired by any customer with the appropriate Specific license. Under a General license these radioactive materials may be acquired only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories and hospitals — and strictly for *in vitro* clinical or laboratory tests not involving external or internal administration of the radioactive material or its radiation to human beings or other animals. Its acquisition, receipt, storage, use, transfer and disposal are all subject to the regulations and a (General or Specific) license of the U.S. Nuclear Regulatory Commission or a State with which the NRC has entered into an agreement for the exercise of regulatory control.

Handle radioactive materials according to the requirements of your General or Specific license. To minimize exposure to radiation, the user should adhere to guidelines set forth in the National Bureau of Standards publication on the *Safe Handling of Radioactive Materials* (Handbook No. 92, issued March 9, 1964) and in subsequent publications issued by State and Federal authorities.

Wipe up spills promptly and decontaminate affected surfaces. Avoid generation of aerosols. Dispose of solid radioactive waste according to license requirements. General licensees (holders of NRC Form 483) may dispose of solid radioactive waste as nonradioactive waste, after removing labeling. Specific licensees (NRC Form 313) should refer to Title 10, Code of Federal Regulations, Part 20. Licensees in Agreement States should refer to the appropriate regulations of their own state. General licensees may dispose of liquid radioactive waste of the type contained in this product through a laboratory sink drain. Licensees must remove or deface labels from empty containers of radioactive materials before disposal of solid waste. Specific licensees may dispose of small quantities of liquid radioactive waste of the type used in this product through a laboratory sink drain. Refer to the appropriate regulations applicable to your laboratory.

Materials Supplied: Initial Preparation

Canine TSH Ab-Coated Tubes (IK91)
Polystyrene tubes coated with murine monoclonal antibodies to canine TSH and packaged in zip-lock bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening: stable at 2–8°C until the expiration date marked on the bag.
IK9T1: 100 tubes.

^{125}I Canine TSH Ab (IK92)
Iodinated anti-cTSH rabbit polyclonal antibody, with preservative. The reagent is supplied in liquid form, ready to use. Each

vial contains 5.5 mL. Stable at 2–8°C for 30 days after opening, or until the expiration date marked on the label.
Color: yellow.
IK9T1: 2 vials.

Canine TSH Calibrators (IK93-9)
Seven vials, labeled A through G, of lyophilized canine TSH calibrators in a cTSH-free canine serum matrix, with preservative. *At least 30 minutes before use,* reconstitute the zero calibrator **A with 2.0 mL** distilled or deionized water, and the remaining calibrators **B through G with 1.0 mL** distilled water. Stable at 2-8°C for 30 days after reconstitution. Stable at –20°C for 6 months.
IK9T1: 1 set.

The calibrators have *lot-specific* values of approximately 0, 0.15, 0.3, 0.6, 1.5, 4.0 and 12 nanograms of canine TSH per milliliter (ng/mL). The assay is standardized in terms of a highly purified preparation of canine pituitary TSH. Intermediate calibration points may be obtained by mixing calibrators in suitable proportions.

Buffered Wash Solution Concentrate (1TSBW)
40 mL of a concentrated buffered saline solution, with surfactants and preservative. Using a transfer container, dilute the vial of concentrate with **400 mL** distilled water, for a total volume of 440 mL. Mix thoroughly before use. Stable at 2–8°C for 6 months after preparation.
IK9T1: 1 vial.

Materials Required But Not Provided

Gamma counter — compatible with standard 12×75 mm tubes.

Rack shaker — set at approximately 200 strokes per minute. Available from DPC as catalog numbers DPSR1 (110 VAC) and DPSR2 (220 VAC).

Reagent Preparation
Distilled or deionized water.

Volumetric pipets: 2 mL and 1 mL.

Graduated cylinder — for dispensing 400 mL.

Plastic storage container with lid — for preparation and storage of Buffered Wash Solution.

Immunoassay
Micropipets: 100 µL.

Dispenser — for delivering 2.0 mL of Buffered Wash Solution. A 2.0 mL dispenser is available from DPC as catalog number DB2ML.

Foam decanting rack — available from DPC.

3-cycle log-log graph paper — available from DPC (catalog number: ZPIRM)

Controls. A bi-level, canine serum-based control, containing cTSH as one of 14 assayed constituents, is available from DPC (catalog number: K9CON). Also available is a bi-level, canine serum-based control module, containing cTSH as one of 3 assayed constituents (catalog number: K9TCM).

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. The time of collection should be noted.

Before assay, allow the samples to come to room temperature (15–28°C) and mix by *gentle* swirling or inversion. Aliquot, if necessary, to avoid repeated thawing and freezing. Do *not* attempt to thaw frozen specimens by heating them in a waterbath.

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. Coat-A-Count Canine TSH IRMA has not been tested with all possible variations of tube types.

Volume Required: 100 µL of serum per tube.

Storage: 2–8°C for 1 week, or for up to 2 months at –20°C.

Immunometric Assay Procedure

All components must be at room temperature (15–28°C) before use.

1 Label fourteen Canine TSH Ab-Coated Tubes A (nonspecific binding) and B through G ("maximum

binding") in duplicate. Label additional Canine TSH Ab-Coated Tubes, also in duplicate, for controls and unknowns.

If Total Counts tubes are required for data reduction, label two plain (uncoated) 12×75 mm polystyrene tubes T (total counts) in duplicate.

Calibrators	Approximate ng/mL
T*	—
A (NSB)	0
B	0.15
C	0.3
D	0.6
E	1.5
F	4.0
G ("MB")	12

*Optional

The values of the canine TSH calibrators are lot-specific. Refer to the calibrator labels for values in ng/mL.

2 Pipet **100 µL** of each calibrator, control and canine serum sample into the tubes prepared.

Pipet directly to the bottom. Samples expected to contain canine TSH concentrations greater than the highest calibrator (approximately 12 ng/mL) should be diluted in the zero calibrator before assay. The use of disposable-tip micropipets is recommended, to avoid carryover from sample to sample. Positive displacement pipets and automatic pipettor-diluters should be used only if the possibility of carryover has been evaluated and found to be insignificant.

3 Add **100 µL** of ^{125}I Canine TSH Ab to every tube.

Pipet directly to the bottom, and make sure that sample and tracer are thoroughly mixed, without foaming. A repeating dispenser is recommended. Set the (optional) T tubes aside for counting at step 6; they require no further processing.

4 Shake at room temperature (15–28°C) for **3 hours** on a rack shaker set at 200 strokes a minute.

5 Decant thoroughly. Add **2 mL** Buffered Wash Solution to each tube. Wait 1 to 2 minutes, then decant thoroughly. Again add **2 mL** Buffered Wash Solution, wait 1 to 2 minutes, and decant thoroughly.

Removing all visible moisture will greatly enhance precision. After the second wash, using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 to 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

6 Count for **1 minute** in a gamma counter.

In multi-head gamma counters, the (optional) Total Counts tubes should be separated from the remaining assay tubes by at least one space, to minimize the possibility of spillover.

Calculation and Quality Control

To calculate results (in terms of concentration units) from a log-log representation of the calibration curve, first correct the counts per minute (CPM) of each pair of tubes by subtracting the average CPM of the nonspecific binding tubes (calibrator A):

Net Counts = (Average CPM) minus (Average **NSB** CPM)

Then determine percent binding (relative to that of the highest calibrator) — here called "%B/MB" — of each pair of tubes as a percent of "maximum binding," with the NSB-corrected counts of the highest calibrator taken as 100%:

Percent Bound = (Net Counts / Net **MB** Counts) × 100

Using 3-cycle log-log graph paper, plot Percent Bound versus Concentration for each of the nonzero calibrators, and draw a curve approximating the path of these points. (Connect the calibration points with arcs or straight line segments. Do not attempt to fit a single straight line to the data.) Concentrations for controls and unknowns within range of the nonzero calibrators may then be estimated from the calibration curve by interpolation. An additional plot of Percent Bound versus Concentration for the three lowest

calibrators on linear-linear graph paper may be used for interpolation near zero dose.

Comments: Although other approaches are acceptable, data reduction by the method just described has certain advantages from the standpoint of quality control. In particular, it yields a calibration curve that is relatively linear in both log-log and linear-linear representations, and relatively stable from assay to assay. It also yields valuable QC parameters, namely, Percent Bound (%B/MB) values for the nonzero calibrators. A still more informative graph, conveying a sense of within-assay reproducibility as a function of concentration, can be obtained by plotting the Percent Bound values of individual calibrator tubes directly, i.e. without first averaging the CPM of replicates.

Alternatives: Although Percent Bound can be calculated directly from Average CPM, correction for nonspecific binding usually produces a calibration curve that is more nearly linear throughout its range. A calibration curve can also be constructed by plotting CPM or Average CPM directly against Concentration on either log-log or linear-linear graph paper. (Semi-log graph paper should *not* be used.) This approach has the virtue of simplicity, but is less desirable from the standpoint of quality control.

Computerized Data Reduction: "Point-to-point" methods, including linear and cubic spline fits, are suitable; but since they provide little assistance in monitoring the integrity of an assay, it is important to prepare the recommended log-log plot of the calibration curve, either manually or by computer, as a quality control step. Data reduction techniques based on the logistic model may also be applicable. Within this family, curve-fitting routines based on the 4- or 5-parameter logistic are the most suitable candidates. However, some algorithms currently in use may not converge successfully, even when the logistic model is true to the data. If a logistic method is adopted, it is essential to verify its appropriateness for each day's assay by monitoring the backcalculation of the calibrators, and other parameters. In addition, a plot of the calibrator curve in a log-log representation is highly recommended, as this is more informative than the conventional semi-log plot.

Sample Handling: The instructions for handling and storing samples and components should be carefully observed. Dilute samples expected to contain canine TSH concentrations greater than the highest calibrator (approximately 12 ng/mL) with the zero calibrator before assay. All samples, including the calibrators and controls, should be assayed at least in duplicate. It is important to use a *disposable-tip* micropipet, changing the tip between samples, in order to avoid carryover contamination. Positive displacement pipets and automatic pipettor-diluters should be used only if the possibility of carryover has been evaluated and found to be insignificant. Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs.

Gamma Counter: To minimize the possibility of spillover in multi-well gamma counters, the optional total counts tubes (T) should be separated by one or more spaces from the other assay tubes. Alternatively, add only 25 µL of the tracer to each of the T tubes at step 3, and multiply the observed counts per minute in these tubes by 4.

Controls: Controls or serum pools with at least two canine TSH concentration levels (low and high) should routinely be assayed as unknowns, and the results charted from day to day as described in Westgard JO, et al. A multi-rule chart for quality control. Clin Chem 1981;27:493-501. Repeat samples are a valuable additional tool for monitoring interassay precision.

Record Keeping: It is good laboratory practice to record for each assay the lot numbers of the components used, as well as the dates when they were first reconstituted or opened.

Further Reading: A technical bulletin titled "Coat-A-Count TSH IRMA: Notes on Data Reduction, QC and Optimization" (catalog number: ZJ019) is available on request. See Dudley RA, et al. Guidelines for immunoassay data reduction. Clin Chem 1985;31:1264-71.

QC Parameters: We recommend keeping track of these performance measures:

T = Total Counts (as counts per minute)

%NSB = 100 × (Average NSB Counts / Total Counts)

%MB = 100 × (Net MB Counts / Total Counts)

And the Percent Bound ("%B/MB") values of all but the highest of the nonzero calibrators, for example:

%C/MB = 100 × (Net Calibrator "C" Counts / Net MB Counts)

Example Run: For illustration only, not for calculating results from another run. Because the calibrator values are *lot-specific*, concentrations listed in the right-most column may not match the values of the calibrators supplied in your shipment. (See “Example Run” table.)

Expected Values

Euthyroidism: Serum samples from a total of 68 dogs with no known thyroid dysfunction were assayed by the Coat-A-Count Canine TSH IRMA procedure. The results showed a median of 0.16 ng/mL, and a 95% reference range of

nondetectable – 0.5 ng/mL

Laboratories should consider these results *as guidelines only.* Each laboratory should establish its own reference ranges.

Performance Data

See Tables and Graphs for data representative of the Coat-A-Count Canine TSH IRMA kit's performance. Results are expressed as nanograms of TSH per milliliter (ng/mL).

Except as noted, all results in the sections below were obtained on canine serum samples.

Calibration Range: 0.15 to 12 ng/mL. Calibrators are *lot-specific*.

Analytical Sensitivity: 0.03 ng/mL.

Intraassay Precision (Within-Run): Statistics were calculated for each of four samples from the results of 20 pairs of tubes in a single assay. (See “Intraassay Precision” table.)

Interassay Precision (Run-to-Run): Statistics were calculated for each of four samples from the results of pairs of tubes in 30 different assays. (See “Interassay Precision” table.)

End-of-Run Effect: None up to approximately 200 tubes. (See "End-of-Run Effect" table.)

Specificity: The Coat-A-Count Canine TSH IRMA antibodies are highly specific for canine TSH, with negligible crossreactivity to related canine pituitary glycoprotein hormones such as FSH and LH.

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

Recovery: Samples spiked 1 to 19 with three TSH solutions (9.8, 39, and 78 ng/mL) were assayed. (See “Recovery” table for representative data.)

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. 7) Ferguson DC. Update on diagnosis of canine hypothyroidism. Vet Clin North Am 1994;24:515-39. 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) Kemppainen 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, 310.645.8200
Fax: 310.645.9999. To place an order:
Tel: 800.372.1782. Fax: 800.234.4372.
Outside the United States, contact your National Distributor.

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

Tables and Graphs

Example Run

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	Approx. Canine TSH ng/mL
T	294,008	295,023			
	296,038				
A (NSB)	187	177	0	—	**0**
	167				
B	948	977	800	**1.6%**	**0.15**
	1,006				
C	1,678	1,664	1,487	**2.9%**	**0.30**
	1,649				
D	3,268	3,227	3,050	**6.0%**	**0.60**
	3,185				
E	8,233	8,284	8,107	**16%**	**1.4**
	8,335				
F	20,534	20,650	20,473	**40%**	**3.9**
	20,766				
G ("MB")	50,818	50,944	50,767	**100%**	**12**
	51,070				
Unknowns					
X1	1,355	1,386	1,209	2.4%	0.24
	1,416				
X2	15,286	15,328	15,151	3.0%	2.8
	15,369				

Quality Control Parameters:
T = 295,023 cpm
%NSB = 0.06%
%MB = 17%
20% Intercept = 1.8 ng/mL
50% Intercept = 5.2 ng/mL
80% Intercept = 9.3 ng/mL

Intraassay Precision (ng/mL)

	Mean	SD	CV
1	0.21	0.02	9.5%
2	0.28	0.01	3.6%
3	0.50	0.02	4.0%
4	3.53	0.07	2.0%

Interassay Precision (ng/mL)

	Mean	SD	CV
1	0.21	0.02	9.5%
2	0.34	0.03	8.8%
3	1.36	0.07	5.1%
4	2.59	0.1	3.9%

End-of-Run Effect (ng/mL).

	Tubes 25 – 30	Tubes 94 – 99	Tubes 163 – 168	Tubes 232 – 237
1	0.21	0.21	0.23	0.25
2	0.65	0.69	0.71	0.68
3	3.70	3.75	3.74	3.94

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	16 in 16	2.24	—	—
	8 in 16	1.11	1.12	99%
	4 in 16	0.55	0.56	98%
	2 in 16	0.28	0.28	100%
	1 in 16	0.14	0.14	100%
2	16 in 16	3.49	—	—
	8 in 16	1.90	1.75	109%
	4 in 16	0.90	0.87	103%
	2 in 16	0.43	0.44	98%
	1 in 16	0.22	0.22	100%
3	16 in 16	4.76	—	—
	8 in 16	2.41	2.38	101%
	4 in 16	1.23	1.19	103%
	2 in 16	0.62	0.60	103%
	1 in 16	0.29	0.30	97%

Recovery (ng/mL)

	Spiking Solution	Observed	Expected	% O/E
1	—	0.24	—	—
	A	0.79	0.72	110%
	B	2.30	2.18	106%
	C	4.40	4.13	107%
2	—	0.63	—	—
	A	1.07	1.09	98%
	B	2.42	2.55	95%
	C	4.31	4.50	96%
3	—	2.70	—	—
	A	3.09	3.06	101%
	B	4.42	4.52	98%
	C	6.32	6.47	98%

Diagnostic Products Corporation
Corporate Offices
5210 Pacific Concourse Drive
Los Angeles, CA 90045-6900
USA

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