COAT-A-COUNT®

Canine T3

DPC®

Coat-A-Count® Canine T3

Intended Use

Coat-A-Count Canine T3 is a solid-phase ^{125}I radioimmunoassay designed for the quantitative measurement of triiodothyronine (T3) in canine serum. It is intended strictly for *in vitro* veterinary use as an aid in the clinical assessment of thyroid status.

Catalog Numbers: **TKC31** (100 tubes), **TKC35** (500 tubes).

The 100-tube kit contains less than 3 microcuries (111 kilobecquerels) of radioactive ^{125}I Canine T3, and the 500-tube kit contains less than 15 microcuries (555 kilobecquerels).

Principle of the Procedure

The Coat-A-Count Canine T3 procedure is a solid-phase radioimmunoassay, in which ^{125}I-labeled T3 competes for a fixed time with T3 in the patient sample for sites on T3-specific antibody. This reaction takes place in the presence of blocking agents which serve to liberate the bound triiodothyronine from carrier proteins; hence the assay measures *total* T3, since both free and protein-bound T3 from the canine sample are able to compete with radiolabeled T3 for antibody sites. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled T3. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the T3 present in the canine sample.

Reagents to Pipet: 1

Total Incubation Time: 2 hours.

Total Counts at Iodination: approximately 50,000 cpm.

Calibration: The kit is equipped with calibrators prepared in charcoal-absorbed *canine* serum. They have *lot–specific* T3 values ranging from approximately 20–600 ng/dL (0.31–9.22 nmol/L). The calibrators are supplied lyophilized for maximum stability.

Warnings and Precautions

For *in vitro* veterinary use.

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 T3 Ab-Coated Tubes (TC31)
Polypropylene tubes coated with antibodies to T3 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. *Color:* pink. These tubes are *not* interchangeable with the tubes supplied in the Coat-A-Count Total T3 kit.
TKC31: 100 tubes. **TKC35:** 500 tubes.

^{125}I Canine T3 (TC32)
Iodinated triiodothyronine with blocking agents for thyroid-binding proteins. Each vial contains 110 mL of liquid reagent, ready to use, with preservative. Stable at 2–8°C for 30 days, or until the expiration date marked on the vial. This tracer is *not* interchangeable with the tracer supplied in the Coat-A-Count Total T3 kit.
TKC31: 1 vial. **TKC35:** 5 vials.

Canine T3 Calibrators (C3D3–8)
Six vials, labeled A through F, of lyophilized processed canine serum, with preservative. At least 30 minutes before use, reconstitute the zero calibrator **A with 2.0 mL** of distilled or deionized water, and each of the remaining calibrators **B through F with 1.0 mL** of distilled or deionized water. Use volumetric pipets and mix by *gentle* swirling. Stable at 2–8°C for 30 days after reconstitution. For longer storage, aliquot and freeze: stable at –20°C for 6 months.
TKC31: 1 set. **TKC35:** 2 sets.

The reconstituted calibrators have *lot–specific* values of approximately 0, 20, 50, 100, 200 and 600 nanograms of T3 per deciliter (ng/dL) in processed canine serum; equivalently: 0, 0.31, 0.77, 1.54, 3.07 and 9.22 nanomoles per liter (nmol/L). Intermediate calibration points can be obtained by mixing calibrators in suitable proportions.

Materials Required But Not Provided

Gamma counter — compatible with standard 12×75 mm tubes

Vortex mixer — recommended

Reagent Preparation
Distilled or deionized water

Volumetric pipets: 1.0 mL and 2.0 mL

Radioimmunoassay
Plain 12×75 mm polypropylene tubes — for use as Total Counts and NSB tubes, available from DPC.

Micropipets: 100 µL and 1,000 µL. For the 1.0 mL reagent addition, a reliable repeating dispenser is also suitable. With the help of an automatic pipettor-diluter, sample and reagent additions may be handled simultaneously.

Waterbath — capable of maintaining 37°C. Neither an oven nor a heat block is suitable.

Controls

Foam decanting rack — available from DPC

Logit-log graph paper — available from DPC (catalog number: ZP797)

Specimen Collection

The animal need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes, and separate serum from cells by centrifugation. The time of collection should be noted.

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

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

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 T3 has not been tested with all possible variations of tube types.

Volume Required: 100 µL of serum.

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.

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

Radioimmunoassay Procedure

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

1 **Plain Tubes:** Label four plain (uncoated) 12×75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

Because nonspecific binding in the Coat-A-Count procedure is characteristically low, the NSB tubes may be safely omitted without detriment to accuracy or quality control.

Coated Tubes: Label twelve Canine T3 Ab-Coated tubes A (maximum binding) and B through F in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and canine samples.

Calibrators	Approx. ng/dL	Approx. nmol/L
A (MB)	0	0
B	20	0.31
C	50	0.77
D	100	1.54
E	200	3.07
F	600	9.22

Note: The values of the calibrators are *lot-specific*. Refer to the calibrator vial labels for values in ng/mL.

2 Pipet **100 µL** of the zero calibrator A into the NSB and A tubes, and 100 µL of each remaining calibrator, control and canine serum sample into the tubes prepared. **Pipet directly to the bottom**.

Canine samples expected to contain T3 concentrations greater than the highest calibrator (600 ng/dL) should be diluted in the zero calibrator before assay.

It is good practice to use a disposable-tip micropipet, changing the tip between samples, in order to avoid carryover contamination.

3 Add **1.0 mL** of ^{125}I Canine T3 to every tube. Vortex briefly and gently.

Laboratories equipped with a reliable pipettor-diluter may handle steps 2 and 3 simultaneously. No more than 10 minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting at step 6; they require no further processing.

4 Incubate for **2 hours at 37°C.**

Use a waterbath; neither an oven nor a heat block is suitable.

5 Decant thoroughly.

Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 or 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.

Calculation of Results

To obtain results in terms of concentration from a logit-log representation of the calibration curve, first calculate for each pair of tubes the average NSB-corrected counts per minute:

$$\text{Net Counts} = \left(\begin{array}{c}\text{Average}\\ \text{CPM}\end{array}\right) \textit{minus} \left(\begin{array}{c}\text{Average}\\ \textbf{NSB}\ \text{CPM}\end{array}\right)$$

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net } \textbf{MB} \text{ Counts}} \times 100$$

(The calculation can be simplified by omitting the correction for nonspecific binding; samples within range of the calibrators yield virtually the same results when Percent Bound is calculated directly from Average CPM.)

Using logit-log graph paper, plot Percent Bound on the vertical (probability) axis against Concentration on the horizontal (logarithmic) axis for each of the nonzero calibrators, and draw a straight line approximating the path of these points. Results for the unknowns may then be read from the line by interpolation.

It is good practice to inspect results for agreement within replicates, and to construct a graph of the calibration curve (even if the calculations are handled by computer) as a visual check on the appropriateness of the transformation used and as a way to detect deviant calibration points. We also recommend keeping track of these data reduction parameters:

T = Total Counts (as counts per minute)

$$\%\text{NSB} = 100 \times \frac{\text{Average NSB Counts}}{\text{Total Counts}}$$

$$\%\text{MB} = 100 \times \frac{\text{Net Counts}}{\text{Total Counts}}$$

And the 20, 50 and 80 percent "intercepts," where

$$20\% = \begin{array}{l}\text{Concentration at}\\ \text{20 Percent Bound, etc.}\end{array}$$

Note that other approaches, e.g. a sound implementation of the 4-parameter logistic, are also acceptable. See Dudley RA, et al. Guidelines for immunoassay data reduction. Clin Chem 1985;31:1264-71.

Example

The values tabulated below are intended for illustration only and should not be used to calculate results from another assay. 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.

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	Approx. T3 ng/dL
T	50,206	50,150			
	50,093				
(NSB)	156	147			
	138				
A (MB)	17,602	17,511	17,364	**100%**	**0**
	17,420				
B	15,832	15,792	15,645	**90.1%**	**20**
	15,751				
C	13,922	13,580	13,433	**77.4%**	**50**
	13,237				
D	11,507	11,321	11,174	**64.4%**	**100**
	11,134				
E	8,372	8,313	8,166	**47.0%**	**200**
	8,254				
F	4,364	4,280	4,133	**23.8%**	**600**
	4,196				
Unknowns:					
X1	11,095	10,874	10,727	61.8%	93
	10,653				
X2	8,732	8,512	8,365	48.2%	197
	8,291				
X3	5,867	5,769	5,622	32.4%	353
	5,670				

Quality Control Parameters:

T = 50,150 cpm
%NSB = 0.3%
%MB = 35%
20% Intercept = 742 ng/dL
50% Intercept = 180 ng/dL
80% Intercept = 44 ng/dL

Quality Control

Controls or canine serum pools with at least two T3 concentration levels — low and high — should routinely be assayed as unknowns.

Report sample results only if the control results for that assay meet your laboratory's established criteria for acceptability.

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. We also recommend charting control results from day to day — as described, for example, in Westgard JO, et al. A multi-rule chart for quality control. Clin Chem 1981;27:493-501. Note that repeat samples can serve as a valuable additional tool for monitoring interassay precision, and that pairs of control tubes can be spaced throughout the assay to help verify the absence of significant drift.

Expected Values

Recent data from several laboratories using the Coat-A-Count Canine T3 kit indicate a suggested reference range for full-grown dogs of

50 – 180 ng/dL (0.77 – 2.79 nmol/L).

These values represent the central 95% of a log-normal distribution with a median of 96 ng/dL. Some care should be exercised in interpreting results in relation to this expected range, which was determined with values obtained from a sample population comprising 51 laboratory and 27 pet dogs with normal T4 levels. It is generally accepted, for example, that kennel or laboratory dogs exhibit lower concentrations of circulating T3 than do pet dogs. Other factors known to depress T3 in dogs include prolonged fasting, starvation, age, certain nonthyroidal illnesses and treatment with various medications. In such cases, a blunted response to a TSH challenge test may aid in confirming a diagnosis of hypothyroidism.

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Performance Data

The following sections contain data *representative* of the Coat-A-Count Canine T3 kit's performance. Canine T3 results in the sections below are expressed as nanograms of T3 per deciliter (ng/dL). To convert to S.I. units — nanomoles per liter (nmol/L), multiply by 0.01536:

ng/dL × 0.01536 → nmol/L

Precision: The reliability of DPC's Coat-A-Count Canine T3 procedure was assessed by examining its reproducibility on samples selected to represent a range of T3 levels.

Intraassay (Within-Run): Statistics were calculated for each of three samples from the results of 20 pairs of tubes in a single assay. Results are expressed as ng/dL.

	Mean	SD	CV
1	92	5.4	5.9%
2	189	9.6	5.1%
3	336	16.9	5.0%

Interassay (Run-to-Run): Statistics were calculated for each of three samples from the results of pairs of tubes in 20 different assays. Results are expressed as ng/dL.

	Mean	SD	CV
1	104	9.4	9.0%
2	211	14.1	6.7%
3	365	23.7	6.5%

References

1) Belshaw BE, Rijnberk A. Radioimmunoassay of plasma T4 and T3 in the diagnosis of primary hypothyroidism in dogs. J Am Animal Hosp Assoc 1979;15:17-23. 2) Ekins RP. Remarks on radioimmunoassay specificity. In: Margoulies M, editor. Proteins and polypeptide hormones. Amsterdam: Excerpta Medica, 1969: 575-81. 3) Kaneko JJ. Thyroid function. In: Kaneko JJ, editor. Clinical biochemistry of domestic animals, 3rd ed. New York: Academic Press, 1980: 491-512. 4) Ferguson DC. Thyroid function tests in the dog. Vet Clin N Amer: Small Anim 1984;14:783-808. 5) Kubasik NP, et al. Clinical evaluation of two thyrotropin radioimmunoassay kits: human serum matrix calibrators and bovine serum matrix calibrators. Clin Chem 1981;27:504-5. 6) Refetoff S. Thyroid function tests. In: L.J. DeGroot, editor. Endocrinology, vol 1. New York: Grune & Stratton, 1979: 387-428. 7) Rosychuk RAW. Thyroid hormones and antithyroid drugs. Vet Clin N Amer: Small Anim 1982;12:111-48. 8) Siegel ET. Endocrine Diseases of the Dog. Philadelphia: Lea & Febiger, 1977: 54-80. 9) Wood WG, et al. A second external quality control survey of serum triiodothyronine (T3) and thyroxine (T4) assays. J Clin Chem Clin Biochem 1980;18:511-9. 10) Clinical endocrinology: update on thyroid testing. Vet Ref Lab Newsletter 1983(Mar/Apr);7(2).

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.

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2005-06-29

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