IMMULITE®

Canine Total T4

For use on the IMMULITE®
and IMMULITE® 1000 systems

DPC®

IMMULITE®/IMMULITE® 1000 Canine Total T4

Intended Use

For *in vitro* use with the IMMULITE and IMMULITE 1000 Analyzers — for the quantitative measurement of total circulating thyroxine (T4) in canine serum. It is intended strictly for *in vitro* veterinary use as an aid in the clinical assessment of thyroid status.

Catalog Numbers: **LKCT1** (100 tests), **LKCT5** (500 tests)

Test Code: **KT4** Color: **Violet**

Summary and Explanation of the Test

Thyroid hormone assays have also proved of value in veterinary medicine.[1,5,14] However, most commercially available T4 RIAs have been designed for measurements in human serum. The reference range for dogs is much lower (approximately 0.73–2.9 µg/dL), with hyperthyroidism characterized by increased levels of circulating T4, hypothyroidism by decreased levels.[17] The differential diagnosis of hypothyroidism is of primary concern, since hyperthyroidism is a rare condition in dogs. The kit is supplied with adjustors prepared in T4-free *canine* serum, to avoid the systematic inaccuracies which can occur due to matrix differences.[3,8,16]

Principle of the Procedure

IMMULITE/IMMULITE 1000 Canine Total T4 is a solid-phase, chemiluminescent competitive immunoassay.

Incubation Cycles: 1 × 30 minutes.

Specimen Collection

The animal need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes (without anticoagulant), and separate the serum from the cells.

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 Total T4 has not been tested with all possible variations of tube types. Consult the section on Gel Barrier Tubes for details on tubes that have been tested.

Volume Required: 30 µL serum. (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 7 days at 2–8°C or 1 month at –20°C.[18] 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.

Warnings and Precautions

For *in vitro* veterinary use only.

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 Total T4 Test Units (LCT1)
Each barcode-labeled unit contains one bead coated with monoclonal murine anti-T4 antibody. Stable at 2–8°C until

expiration date.
LKCT1: 100 units. **LKCT5:** 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 Total T4 Reagent Wedge (LCT2)
With barcode. 7.5 mL alkaline phosphatase (bovine calf intestine) conjugated to T4 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.
LKCT1: 2 wedges. **LKCT5:** 10 wedges.

Canine Total T4 Adjustors (LCTL, LCTH)
Two vials (Low and High) of lyophilized T4 in processed canine serum, with preservative. *At least 30 minutes before use*, reconstitute each vial with **2.0 mL** distilled or deionized water. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

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 T4 as one of multiple assayed constituents.

K9TCM: A bi-level, canine serum-based control module, containing canine T4 as one of 3 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 T4.

Expected Values

A reference range study performed with the IMMULITE Canine Total T4 kit on a total of 46 apparently healthy dogs yielded a median of 2.2 µg/dL (28 nmol/L) and a range of

1.3 to 2.9 µg/dL (17 to 37 nmol/L).

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

Performance Data

Results are expressed in µg/dL. (Unless otherwise specified, all results were generated on canine samples collected in tubes without anticoagulants, gel barriers, or clot-promoting additives.)

Conversion Factor:
µg/dL × 12.87 → nmol/L

Calibration Range: 0.5 – 15 µg/dL (6.4 – 193 nmol/L).

Sensitivity: 0.12 µg/dL (1.5 nmol/L).

Precision: Samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates (µg/dL).

		Within-Run		Total	
	Mean	SD	CV	SD	CV
1	0.65	0.07	10.8%	0.09	13.8%
2	0.83	0.08	9.6%	0.11	13.2%
3	1.95	0.1	5.1%	0.16	8.2%
4	3.84	0.17	4.4%	0.26	6.8%
5	6.11	0.29	4.7%	0.35	5.7%
6	11.9	0.47	3.9%	0.62	5.2%

Linearity: Samples were assayed under various dilutions.

	Dilution	Observed µg/dL	Expected µg/dL	%O/E
1	4 in 4	1.8	—	—
	2 in 4	0.95	0.90	106%
	1 in 4	0.52	0.45	116%
2	4 in 4	2.2	—	—
	2 in 4	1.2	1.1	109%
	1 in 4	0.59	0.55	107%
3	4 in 4	2.8	—	—
	2 in 4	1.5	1.4	107%
	1 in 4	0.64	0.70	91%
4	4 in 4	6.2	—	—
	2 in 4	3.2	3.1	103%
	1 in 4	1.6	1.6	100%

Recovery: Samples spiked 1 to 19 with three T4 solutions (17, 32 and 62 µg/dL) were assayed.

	Solution[1]	Observed µg/dL	Expected µg/dL	%O/E
1	—	0.57	—	—
	A	1.5	1.4	107%
	B	2.4	2.1	114%
	C	4.1	3.6	114%
2	—	1.1	—	—
	A	1.9	1.9	100%
	B	2.8	2.6	108%
	C	4.6	4.1	112%
3	—	1.2	—	—
	A	2.1	2.0	105%
	B	2.8	2.7	104%
	C	4.6	4.2	110%
4	—	1.8	—	—
	A	2.5	2.6	96%
	B	3.3	3.3	100%
	C	4.9	4.8	102%

Specificity: The antibody used in the IMMULITE Canine Total T4 procedure is highly specific for T4, with low crossreactivity to other compounds and therapeutic drugs that may be present in canine samples.

Compound	µg/dL Added	Apparent µg/dL	% Cross-reactivity
L-Thyroxine (T4)	—	—	100%
D-Thyroxine	10	5.5	55%
Tetraiodothyroacetic acid	10	1.6	16%
Triiodo-L-thyronine	100	3.2	3.2%
	25	1.1	4.4%
Triiodo-D-thyronine	1	ND	ND
Triiodothyroacetic acid	1,000	6.3	0.63%
	10	ND	ND
Monoiodotyrosine	1,000	1.6	0.16%
	10	ND	ND
Diiodo-L-tyrosine	1,000	ND	ND
Methimazole	1,000	ND	ND
5,5′-Diphenylhydantoin	1,000	ND	ND
Phenylbutazone	1,000	ND	ND
6-*n*-Propyl-2-thiouracil	1,000	ND	ND

ND: not detectable.

Effect of Gel Barrier Tubes: Blood was collected from 8 dogs into plain and Becton Dickinson SST® vacutainer tubes. The samples were processed by the IMMULITE Canine Total T4 procedure, with the following results.

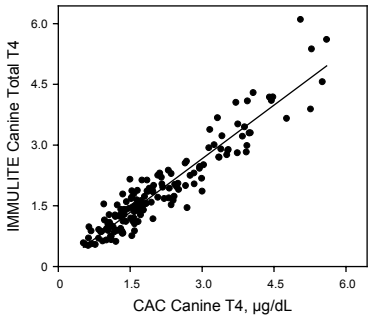
(SST) = 0.92 (Plain Tubes) + 0.06 µg/dL
r = 0.985

Means:
0.59 µg/dL (Plain Tubes)
0.60 µg/dL (SST Tubes)

Method Comparison: The assay was compared to DPC's Coat-A-Count Canine T4 assay on 154 canine samples. (Concentration range: approximately 0.53 to 5.6 µg/dL. See graph.) By linear regression:

(IMMULITE) = 0.88 (CAC) + 0.01 µg/dL
r = 0.942

Means:
1.9 µg/dL (IMMULITE)
2.1 µg/dL (Coat-A-Count)



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Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
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