Quantikine® ELISA

Canine IL-10 Immunoassay

Catalog Number CA1000

For the quantitative determination of canine Interleukin 10 (IL-10) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-3). IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Mature canine IL-10 shares 85-86% amino acid (aa) sequence identity with equine and feline IL-10 and 72%-80% with bovine, guinea pig, human, mouse, ovine, porcine, and rat IL-10. It contains two intrachain disulfide bridges and is expressed as a 36 kDa noncovalently-associated homodimer (4-6,).

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells (7-12), while the 75 kDa transmembrane IL-10 Rβ is widely expressed (13, 14). The IL-10 dimer binds to two IL 10 Rα chains, triggering recruitment of two IL-10 Rβ chains (13, 14). IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (15-17).

The involvement of IL-10 in immunoregulation includes both suppressive and stimulatory effects. It functions as an anti-inflammatory cytokine by inhibiting the expansion and activation of Th1 cells and Th17 cells (18-20) and by promoting the development of M2 macrophages (20). Its expression by immunosuppressive regulatory T cells (Treg) and regulatory B cells is important for Treg proliferation (18). Within a tumor microrenvironment, however, IL-10 inhibits the expansion of Treg as well as myeloid-derived suppressor cells (21, 22). IL-10 induces the intratumoral accumulation and activation of $CD8^+$ T cells (23, 24). IL-10 exerts protective effects including limiting tissue damage in arthritic inflammation (18) and promoting muscle regeneration after injury (20), but it also contributes to the persistence of viral infections (25). The levels of IL-10 are elevated in Sjogren's syndrome (saliva), primary CNS lymphoma (cerebrospinal fluid), and ovarian cancer (serum and ascites) (26-28). Its levels are decreased in the serum in patients with recurrent heart attacks or during preeclampsia and also in the seminal fluid of infertile men (29-31).

The Quantikine® Canine IL-10 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure canine IL-10 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant canine IL-10 and antibodies raised against the recombinant protein. Results obtained for naturally occurring canine IL-10 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural canine IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for canine IL-10 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for canine IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-10 bound in the initial step. The sample values are then read from the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Canine IL-10 Microplate	892492	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for canine IL-10.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Canine IL-10 Standard	892494	2 vials of recombinant canine IL-10 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Canine IL-10 Control	892495	2 vials of recombinant canine IL-10 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Canine IL-10 Conjugate	892493	12 mL of a monoclonal antibody specific for canine IL-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-14	895180	12 mL of a buffered protein base with preservatives. *Contains a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6-12	895214	21 mL of animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 30 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 30 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Cell culture supernates, serum, and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD6-12.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

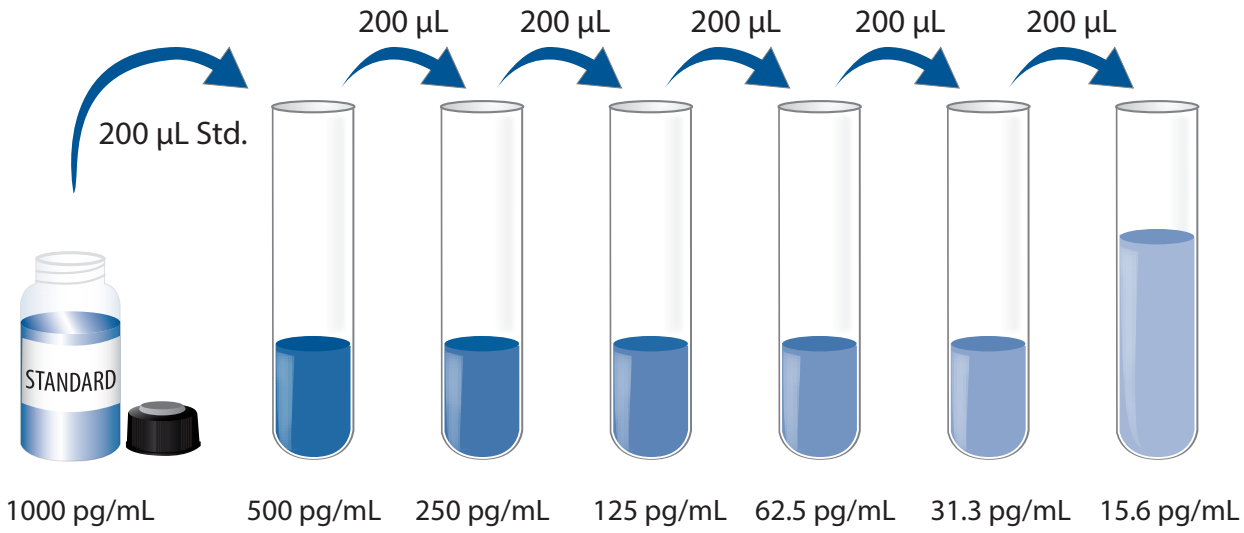
Canine IL-10 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Canine IL-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Canine IL-10 Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Canine IL-10 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD6-12 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-14 to each well. *Assay Diluent RD1-14 contains a precipitate. Mix well before and during its use.*
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Canine IL-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

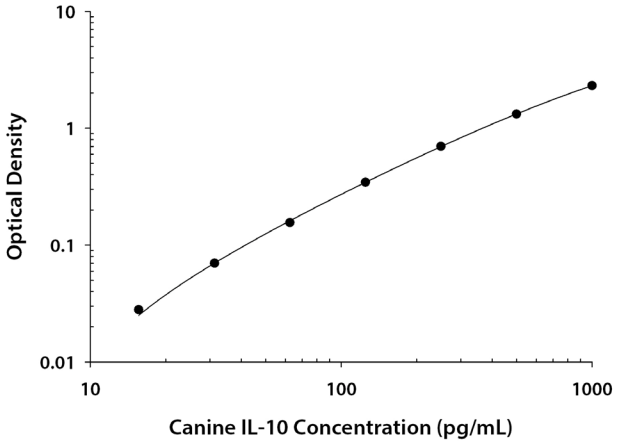
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the canine IL-10 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.046 0.047	0.046	—
15.6	0.074 0.075	0.074	0.028
31.3	0.114 0.118	0.116	0.070
62.5	0.193 0.211	0.202	0.156
125	0.368 0.414	0.391	0.345
250	0.708 0.783	0.746	0.700
500	1.298 1.439	1.368	1.322
1000	2.347 2.373	2.360	2.314

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	53	144	533	59	152	512
Standard deviation	2.9	7.0	20.1	5.1	9.3	21.0
CV (%)	5.5	4.9	3.8	8.6	6.1	4.1

RECOVERY

The recovery of canine IL-10 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture supernate (n=5)	93	75-104%
Serum (n=5)	96	80-107%
EDTA plasma (n=4)	89	83-93%
Heparin plasma (n=4)	87	83-93%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of canine IL-10 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture samples (n=7)	Serum (n=4)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	95	108	106	105
	Range (%)	89-99	106-110	102-113	102-109
1:4	Average % of Expected	95	110	108	105
	Range (%)	88-98	108-116	104-117	100-111
1:8	Average % of Expected	96	111	106	107
	Range (%)	89-101	109-114	102-114	105-114
1:16	Average % of Expected	97	115	105	111
	Range (%)	92-106	112-119	99-110	108-113

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of canine IL-10 ranged from 1.0-3.8 pg/mL. The mean MDD was 2.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant canine IL-10 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of canine IL-10 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=10)	43	20	ND-52
EDTA Plasma (n=10)	56	30	ND-85
Heparin Plasma (n=10)	53	30	ND-65

ND=Non-detectable

Cell Culture Supernates - Canine peripheral blood cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum and stimulated with 1 µg/mL LPS and 10^{-5} M histamine for 48 hours. An aliquot of the cell culture supernate was removed, assayed for canine IL-10, and measured 250 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant canine IL-10.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range canine IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant canine:
IFN-γ
IL-4

Recombinant human:
IFN-γ
IL-4
IL-10

Recombinant mouse:
IFN-γ
IL-4
IL-10

Recombinant rat:
IFN-γ
IL-4
IL-10

Recombinant porcine:
IFN-γ
IL-4
IL-10

Recombinant viral:
IL-10 HCMV
IL-10 EBV

Other recombinants:
cotton rat IL-10
feline IL-10

Recombinant human and mouse IL-10 interfere at concentrations > 25 ng/mL.

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