

REFERENCES

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3. Lin, P.L. et al. (2007) J Investig Dermatol Symp Proc 12, 22-5.
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Canine IL-6 Immunoassay

Catalog Number: SEKC-0021

For the quantitative determination of canine IL-6 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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TABLE OF CONTENTS

SECTION	PAGE
BACKGROUND.....	01
PRINCIPLE OF THE ASSAY.....	01
TECHNICAL HINTS AND LIMITATIONS.....	02
PRECAUTIONS.....	02
KIT COMPONENTS& STORAGE CONDITIONS.....	03
OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED.....	04
SPECIMEN COLLECTION & STORAGE.....	04
REAGENTS PREPARATION.....	04
ASSAY PROCEDURE.....	06
CALCULATION OF RESULTS.....	07
PERFORMANCE CHARACTERISTICS.....	08
REFERENCES.....	10

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

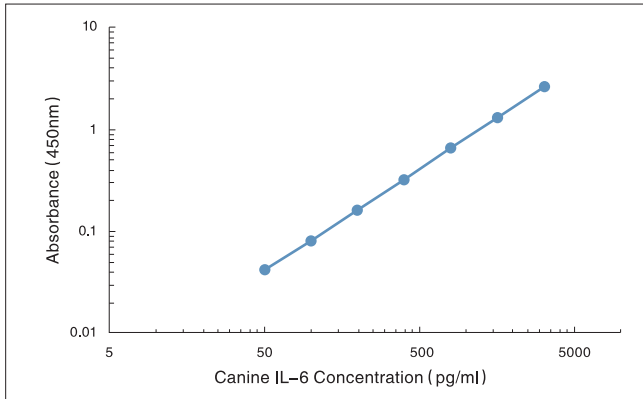
RECOVERY: The recovery of IL-6 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IL-6 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	93	85-102
Cell culture supernatants	96	88-104

LINEARITY:To assess the linearity of the assay, three samples were spiked with high concentrations of IL-6 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	94	104
	Range (%)	85-102	96-113
1:4	Average% of Expected	95	106
	Range (%)	87-105	96-117



Representative standard curve for IL-6 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 10 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant canine IL-6. The factors listed below were prepared at 10ng/ml in Standard/sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

BMP1, BMP2, BMP4, HGF, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, sIL-6R, IL-8, IL-10, IL-12, IL-13, IL-15, IFN γ , MMP-2, MMP9, IL2R, TGF β 1, TGF β 2, TGF β 3, TLR1, TLR2, TLR3, TNF- α , TNF RI, TNF RII, VEGF.

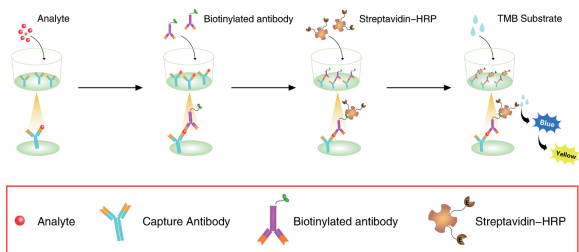
BACKGROUND

IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine and is produced by T cells, macrophages, fibroblasts, osteoblasts, endothelial and other cells. IL-6 induces proliferation and differentiation and acts on B cells, T cells, thymocytes, and others. IL-6 is one of the most important mediators of fever and of the acute phase response. In the muscle and fatty tissue, IL-6 stimulates energy mobilization that leads to increased body temperature. IL-6 can be secreted by macrophages in response to specific microbial molecules, referred to as pathogen associated molecular patterns (PAMPS). IL-6 in concert with TGF β is important for developing Th17 responses. IL-6 binds to IL-6R α that through association induces gp130 homodimerization. gp130 homodimerization triggers the Jak/STAT cascade and the SHP2/Erk Map kinase cascade. IL-6 also forms a complex with an IL-6R α splice variant that is non-membrane associated. The IL-6/soluble IL-6R α complex can then activate the gp130 signaling pathway on cells that express gp130 but not IL6R α . IL-6 is relevant to many disease processes such as diabetes, atherosclerosis, depression, Alzheimer's Disease, systemic lupus erythematosus, prostate cancer, breast cancer, and rheumatoid arthritis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal β antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IL-6 is added to detect the captured IL-6 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

DESCRIPTION



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

DESCRIPTION

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the IL-6 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IL-6 ELISA

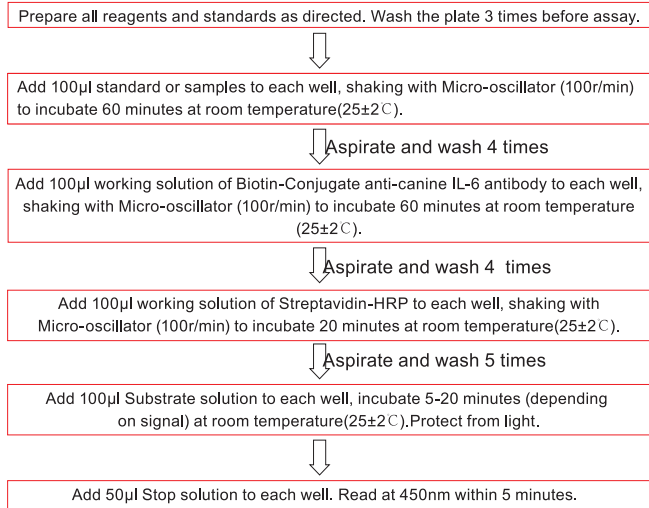
Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.062	0.063	0.062	-----
50	0.083	0.092	0.087	0.025
100	0.176	0.18	0.178	0.115
200	0.287	0.321	0.304	0.241
400	0.512	0.534	0.523	0.460
800	0.834	0.861	0.847	0.785
1600	1.567	1.543	1.555	1.492
3200	2.356	2.385	2.370	2.308

DESCRIPTION

5. **Working solution of Streptavidin-HRP(120µL)**-Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 µL HRP Conjugate sufficient for 96-well plate. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 ul of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE



DESCRIPTION

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells x12 strips)	1 plate	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**
Standard-lyophilized,3200pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C **for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C **for six months
Substrate Solution	1 bottle	Store at 2-8°C **for six months
Stop Solution	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 15 minutes at 1000×g. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

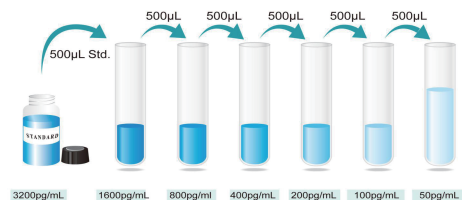
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: The normal canine serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer**- Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Specimen (2vials)**- canine IL-6 Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of Standard /sample

Diluent. This reconstitution produces a stock solution of 3200 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard /sample Diluent into 1600pg/ml tube and the remaining tubes. Use the stock solution of 3200pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 3200 pg/mL standard serves as the high standard. The Standard /sample Diluent serves as the zero standard (0 pg/mL).



Preparation of canine IL-6 standard dilutions

***If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-canine IL-6 antibody(1vials)** -The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**