Quantikine® ELISA

Porcine IL-6 Immunoassay

Catalog Number P6000B

For the quantitative determination of porcine interleukin 6 (IL-6) concentrations in cell culture supernates, serum, and plasma.

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

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic α-helical 22-28 kDa phosphorylated and variably glycosylated (1-5) cytokine that plays important roles in acute phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression. Mature porcine IL-6 is 183 amino acids in length and shares 58% and 39% amino acid (aa) identity with human and mouse IL-6, respectively (6, 7). Porcine IL-6 is reported to be active on both human and mouse cells (7, 8). In humans, alternate splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties (9-12). Cells known to express IL-6 include $CD8^+$ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells, and pancreatic islet beta cells (2, 9, 12-35). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2). In humans, circulating IL-6 shows slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (36-40).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R) and a signal transducing subunit (gp130). IL-6 binds to IL-6 R, triggering IL-6 R association with gp130 and gp130 dimerization (41). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (42). Soluble forms of IL-6 R are generated by both alternate splicing and proteolytic cleavage (3). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 R elicit responses from gp130-expressing cells that lack cell surface IL-6 R (1, 3). Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 R is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (1-3). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 R but not from other cytokines that use gp130 as a co-receptor (3, 43).

IL-6 drives the acute inflammatory response along with TNF-α and IL-1 and is almost solely responsible for fever and the acute phase response in the liver. It is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-4). IL-6 contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, inflammatory arthritis, and sepsis when dysregulated, often involving IL-6 trans-signaling (1, 2). It plays an important role in differentiation of naïve T cells to Th17 inflammatory cells in the presence of TGF-β. IL-6 modulates bone resorption and is a major effector in inflammatory joint destruction in rheumatoid arthritis through promoting Th17 T cell activity (1). It contributes to atherosclerotic plaque development and destabilization (2). However, IL-6 can also have anti-inflammatory effects, such as in skeletal muscle where it is secreted in response to exercise (2). It promotes hematopoiesis by being a growth factor for hematopoietic stem cells, induces B cell maturation to plasma cells, and perpetuates multiple myeloma (1, 44). IL-6 also promotes, but probably does not initiate, other types of inflammation-associated carcinogenesis, such as colitis-associated cancer (1).

The Quantikine Porcine IL-6 Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-6 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant porcine IL-6 and antibodies raised against the recombinant factor. The immunoassay has been shown to accurately quantitate recombinant porcine IL-6. Results obtained using natural porcine IL-6 show dose response curves that were parallel to the standard curves obtained using the recombinant standards. These results indicate that this kit can be used to determine relative mass values for natural porcine IL-6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for porcine IL-6 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any porcine IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for porcine IL-6 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 porcine IL-6 bound in the initial step. The sample values are then read off 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, 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
Porcine IL-6 Microplate	893847	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for porcine IL-6.	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.*
Porcine IL-6 Standard	893849	2 vials (2.4 ng/vial) of recombinant porcine IL-6 in a buffered protein base with preservatives; lyophilized.	Use a new Standard and Control for each assay.
Porcine IL-6 Control	893850	2 vials of recombinant porcine IL-6 in a buffered protein base with preservatives; lyophilized. The concentration range of porcine IL-6 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Porcine IL-6 Conjugate	893848	12 mL of a polyclonal antibody against porcine IL-6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD6-31	895323	21 mL of diluted 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.

PRECAUTIONS

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

Some components in this kit contain ProClin® 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. Please 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 20 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 20 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

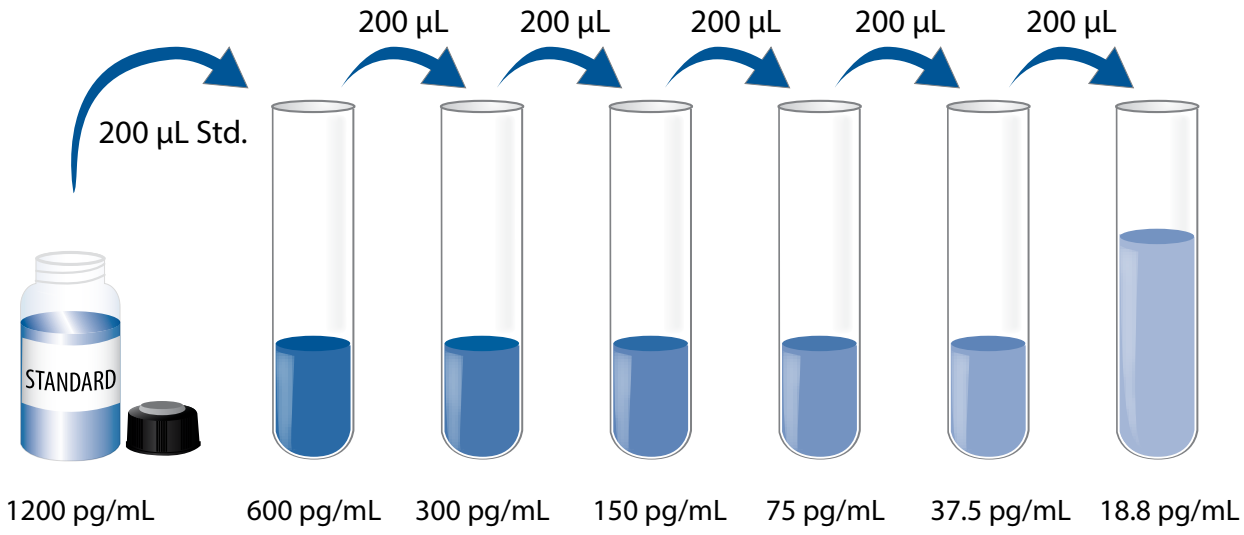
Porcine IL-6 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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.

Porcine IL-6 Standard - Reconstitute the Porcine IL-6 Standard with 2.0 mL of Calibrator Diluent RD6-31. Do not substitute other diluents. This reconstitution produces a stock solution of 1200 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions. Mix each tube thoroughly before the next transfer.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD6-31 into each tube. Use the stock solution to produce a dilution series (below). The undiluted Porcine IL-6 Standard serves as the high standard (1200 pg/mL). Calibrator Diluent RD6-31 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 reagents, samples, standards, and control as directed by 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 RD1-63 to each well.
4. Add 50 µL of Standard, Control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
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 Porcine IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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.

CALCULATION OF RESULTS

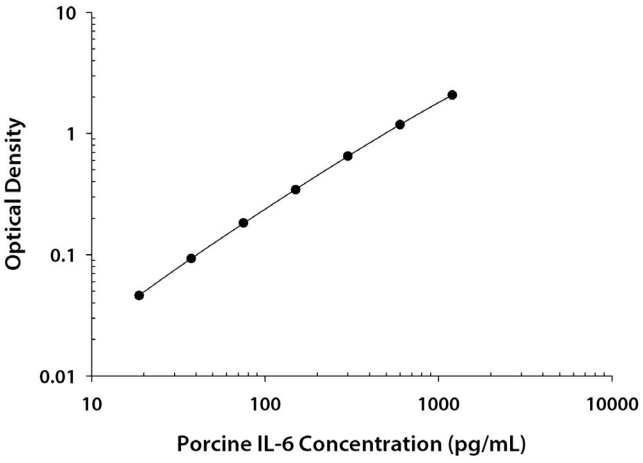
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 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.

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	0.064 0.066	0.065	—
18.8	0.109 0.113	0.111	0.046
37.5	0.152 0.163	0.158	0.093
75	0.243 0.252	0.248	0.183
150	0.406 0.412	0.409	0.344
300	0.710 0.721	0.716	0.651
600	1.226 1.271	1.249	1.184
1200	2.140 2.141	2.141	2.076

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 forty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	54.5	123	674	52.9	127	665
Standard deviation	2.8	5.4	17.6	3.9	7.5	30.1
CV (%)	5.1	4.4	2.6	7.4	5.9	4.5

RECOVERY

The recovery of porcine IL-6 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	102	95-109%
Serum (n=4)	96	87-110%
EDTA plasma (n=4)	97	89-104%
Heparin plasma (n=4)	97	85-103%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of porcine IL-6 in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	99	96	96	91
	Range (%)	98-101	91-100	92-99	89-93
1:4	Average % of Expected	100	100	96	97
	Range (%)	96-103	93-104	93-97	94-101
1:8	Average % of Expected	100	105	97	103
	Range (%)	94-104	96-115	94-100	95-108
1:16	Average % of Expected	104	109	104	110
	Range (%)	100-108	97-118	101-108	99-120

SENSITIVITY

Sixty-eight assays were evaluated and the minimum detectable dose (MDD) of porcine IL-6 ranged from 0.68-4.30 pg/mL. The mean MDD was 2.03 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant porcine IL-6 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of porcine IL-6 in the assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=17)	38	41	ND-69
EDTA plasma (n=17)	39	12	ND-52
Heparin plasma (n=10)	42	20	ND-58

ND=Non-detectable

Cell Culture Supernates:

Porcine peripheral blood lymphocytes cells (PBL; 20 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate and stimulated with 100 ng/mL lipopolysaccharide (LPS) for 18 hours. The cell culture supernate was then removed. Cells were resuspended in fresh media containing 100 ng/mL LPS, and incubated for 3 days. An aliquot of the cell culture supernate was removed, assayed for levels of porcine IL-6, and measured 895 pg/mL.

Porcine peripheral blood lymphocytes cells (PBL; 20 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate. The cell culture supernate was unstimulated or stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin for 18 hours or 4 days. Aliquots of the cell culture supernates were removed and assayed for levels of porcine IL-6.

Sample Type	18 hours (pg/mL)	Day 4 (pg/mL)
PBL Unstimulated	ND	ND
PBL Stimulated with PMA + Ca^{2+}	289	80.7

ND=Non-detectable

SAMPLE VALUES *CONTINUED*

PK-15 porcine kidney epithelial cells (0.5 x 10^5 cells/mL) were cultured in MEM NEAA supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was unstimulated or stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin or 100 ng/mL LPS for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of porcine IL-6.

Sample Type	(pg/mL)
PK-15 Unstimulated	ND
PK-15 Stimulated with LPS	ND
PK-15 Stimulated with PMA + Ca^{2+}	1969

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant porcine IL-6.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range porcine IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:

GM-CSF
IL-1α
IL-1β
IL-1ra
IL-2
IL-4
IL-8
IL-10
IL-12
TNF-α

Recombinant human:

gp130
IL-6 Rα
IL-6 Rα/gp130

Other recombinants:

cotton rat IL-6
equine IL-6
mouse IL-6
rat IL-6

Natural proteins:

porcine PDGF
porcine TGF-β1

Recombinant human IL-6 cross-reacts approximately 0.05% in this assay.

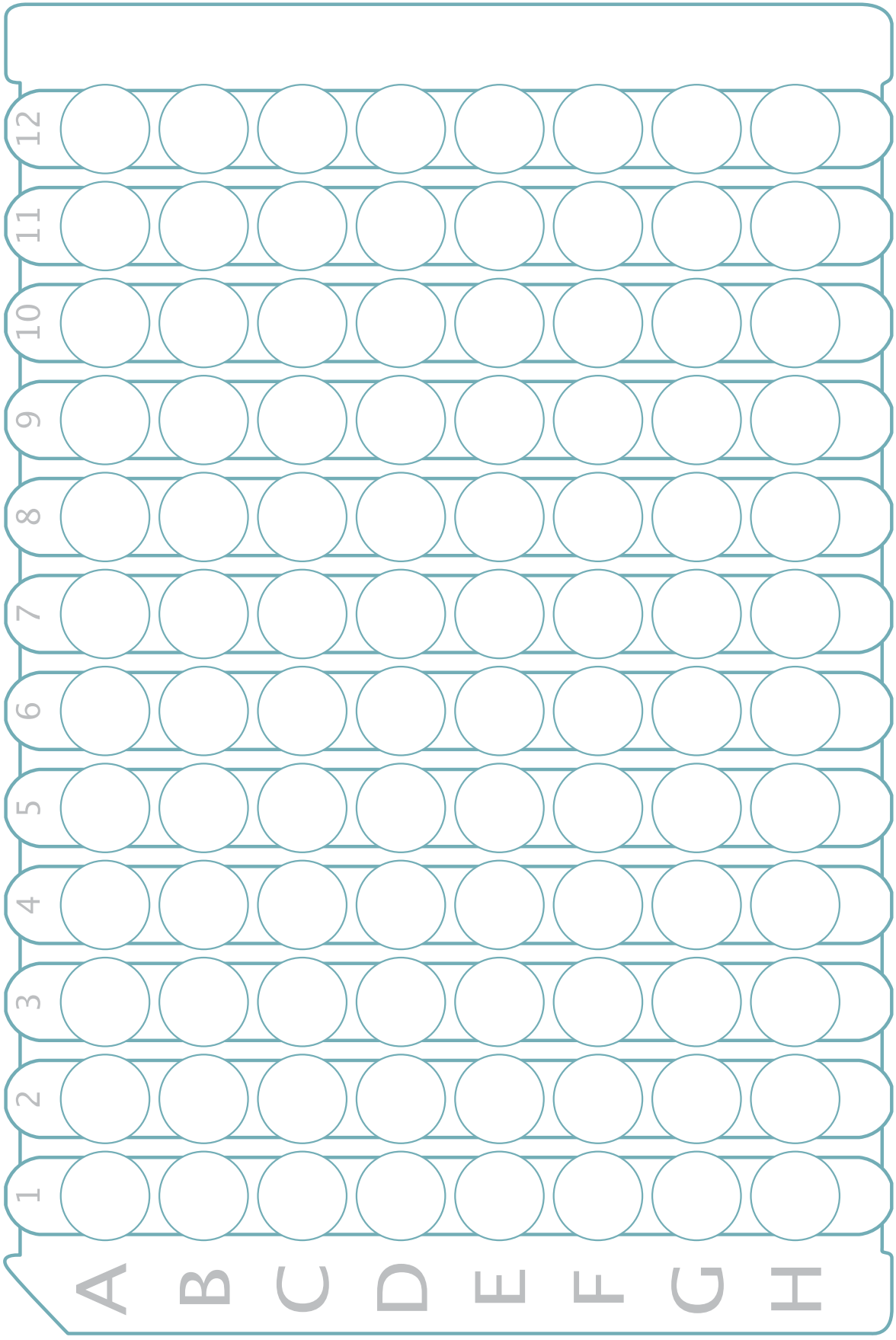
Recombinant feline IL-6 cross-reacts approximately 0.12% in this assay.

REFERENCES

1. Naugler, W.E. and M. Karin (2008) Trends Mol. Med. **14**:109.
2. Schuett, H. *et al.* (2009) Thromb. Haemost. **102**:215.
3. Jones, S.A. (2005) J. Immunol. **175**:3468.
4. Hodge, D.R. *et al.* (2005) Eur. J. Cancer **41**:2502.
5. Rose-John, S. *et al.* (2006) J. Leukoc. Biol. **80**:227.
6. Richards, C.D. and J. Saklatvala (1991) Cytokine **3**:269.
7. Mathialagan, N. *et al.* (1992) Mol. Reprod. Dev. **32**:324.
8. Anegon, I. *et al.* (1994) Cytokine **6**:493.
9. Kestler, D.P. *et al.* (1995) Blood **86**:4559.
10. Kestler, D.P. *et al.* (1999) Am. J. Hematol. **61**:169.
11. Bihl, M.P. *et al.* (2002) Am. J. Respir. Cell Mol. Biol. **27**:48.
12. Alberti, L. *et al.* (2005) Cancer Res. **65**:2.
13. May, L.T. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:8957.
14. Sad, S. *et al.* (1995) Immunity **2**:271.
15. Cichy, J. *et al.* (1996) Biochem. Biophys. Res. Commun. **227**:318.
16. Miyazawa, K. *et al.* (1998) Am. J. Pathol. **152**:793.
17. Fried, S.K. *et al.* (1998) Endocrinology **83**:847.
18. Ishimi, Y. *et al.* (1990) J. Immunol. **145**:3297.
19. Jiang, S. *et al.* (1994) Blood **84**:4151.
20. Xin, X. *et al.* (1995) Endocrinology **136**:132.
21. Marz, P. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:3251.
22. Ringheim, G.E. *et al.* (1995) J. Neuroimmunol. **63**:113.
23. Gadient, R.A. *et al.* (1995) Neurosci. Lett. **194**:17.
24. Kuppner, M.C. *et al.* (1995) Immunology **84**:265.
25. Gagari, E. *et al.* (1997) Blood **89**:2654.
26. Cumberbatch, M. *et al.* (1996) Immunology **87**:513.
27. Fujisawa, H. *et al.* (1997) J. Interf. Cytokine Res. **17**:347.
28. Lee, S.C. *et al.* (1993) J. Immunol. **150**:2659.
29. Lafortune, L. *et al.* (1996) J. Neuropathol. Exp. Neurol. **55**:515.
30. Ericson, S.G. *et al.* (1998) Blood **91**:2099.
31. Melani, C. *et al.* (1993) Blood **81**:2744.
32. Lacy, P. *et al.* (1998) Blood **91**:2508.
33. Jung, H.C. *et al.* (1995) J. Clin. Invest. **95**:55.
34. Spencer, N.F.L. and R.A. Daynes (1997) Int. Immunol. **9**:745.
35. Campbell, I.L. *et al.* (1989) J. Immunol. **143**:1188.
36. D'Auria, L. *et al.* (1997) Eur. Cytokine Netw. **8**:383.
37. Yamamura, M. *et al.* (1998) Br. J. Haematol. **100**:129.
38. Angstwurm, M.W.A. *et al.* (1997) Cytokine **9**:370.
39. Mouawad, R. *et al.* (1996) Clin. Cancer Res. **2**:1405.
40. Sakamoto, K. *et al.* (1994) Cytokine **6**:181.
41. Murakami, M. *et al.* (1993) Science **260**:1808.
42. Muller-Newen, G. (2003) Sci. STKE **2003**:PE40.
43. Mitsuyama, K. *et al.* (2006) Clin. Exp. Immunol. **143**:125.
44. Cerutti, A. *et al.* (1998) J. Immunol. **160**:2145.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES