Quantikine™ ELISA

Mouse IL-6 Immunoassay

Catalog Number M6000B-1
SM6000B
PM6000B

For the quantitative determination of mouse 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

Manufactured and Distributed by:

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

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic, α-helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature mouse IL-6 is 187 amino acids (aa) in length and shares 39% and 85% aa sequence identity with human and rat IL-6, respectively (6-8). 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, 9-31). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 Rα) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization (37). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (38). Soluble forms of IL-6 Rα are generated by both alternative splicing and proteolytic cleavage (5). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 Rα elicit responses from gp130-expressing cells that lack cell surface IL-6 Rα (5). 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 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα but not from other cytokines that use gp130 as a co-receptor (5, 39).

IL-6, along with TNF-α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-5). When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis (2, 5). IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity (1). It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis (1, 2). IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise (2). In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells (40).

The Quantikine™ Mouse IL-6 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-6 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-6 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse IL-6. Results obtained using natural mouse IL-6 showed dose response 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 mouse IL-6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-6 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse 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 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 operator, pipetting and 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 #	CATALOG # M6000B-1	CATALOG # SM6000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-6 Microplate	892369	1 plate	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse 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.*
Mouse IL-6 Standard	892371	1 vial	3 vials	Recombinant mouse IL-6 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze thaw cycles.
Mouse IL-6 Control	892372	1 vial	6 vials	Recombinant mouse IL-6 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	Aliquot and store for up to 2 weeks at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze thaw cycles.
Mouse IL-6 Conjugate	899292	1 vial	6 vials	12.5 mL/vial of a polyclonal antibody specific for mouse IL-6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-14	895180	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives. *Contains a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5T	895175	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

M6000B-1 contains sufficient materials to run ELISAs on one 96 well plate.
SM6000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM6000B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Mouse IL-6 Microplate	892369	50 plates
Mouse IL-6 Conjugate	899292	50 vials
Mouse IL-6 Standard *	892371	25 vials
Mouse IL-6 Control	892372	25 vials
Assay Diluent RD1-14	895180	25 vials
Calibrator Diluent RD5T	895175	25 vials
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895174	25 vials
Plate sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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

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 SDS 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 and 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 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA and citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

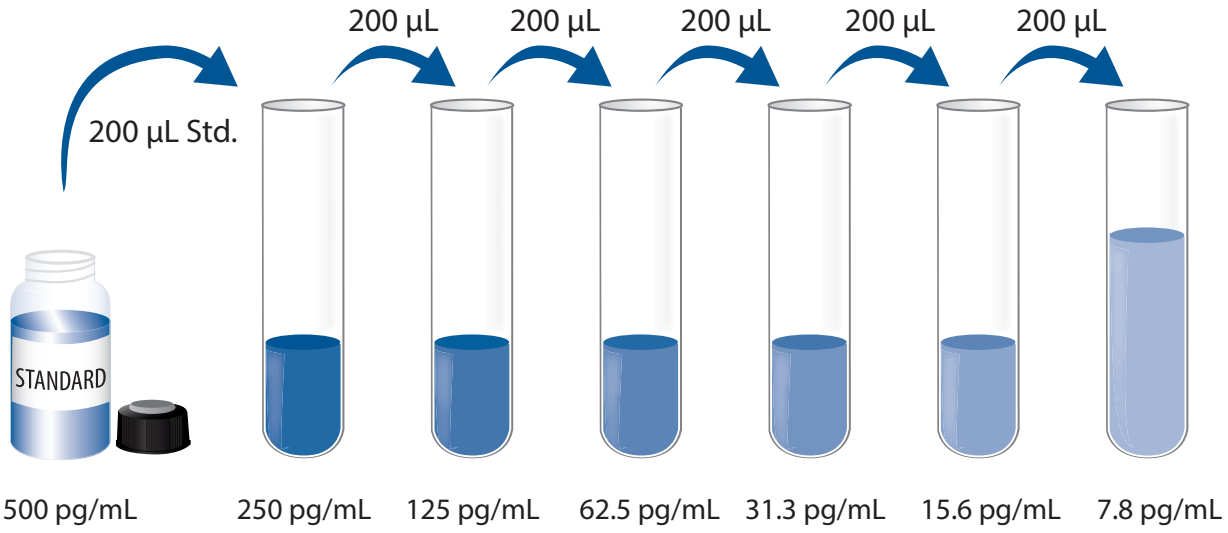
Mouse IL-6 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Assay the control undiluted. Mix thoroughly.

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 into 480 mL of 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. 100 µL of the resultant mixture is required per well.

Mouse IL-6 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-6 Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The Mouse IL-6 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5T 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, and standard dilutions 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 RD1-14 to the center of each well. *RD1-14 contains undissolved material. Mix well before and during its use.*
4. Add 50 µL of standard, control, or sample to the center of each well. Cover with the adhesive strip provided. Mix by gently tapping the plate frame for 1 minute. Incubate for 2 hours at room temperature. A plate layout is provided as a record of samples and standards 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 Mouse IL-6 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.

CALCULATION OF RESULTS

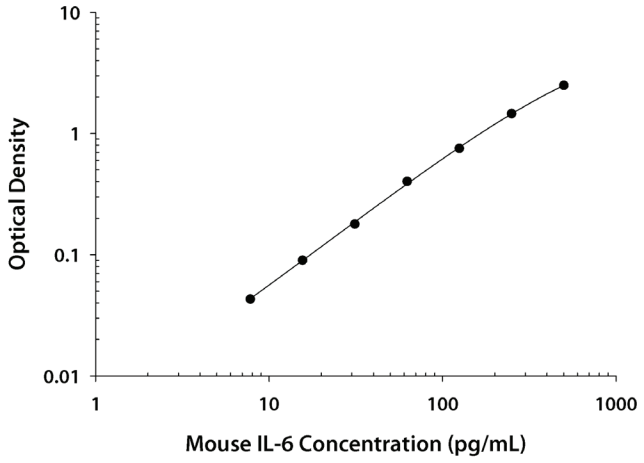
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse 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	0.010 0.010	0.010	—
7.8	0.052 0.054	0.053	0.043
15.6	0.098 0.101	0.100	0.090
31.3	0.181 0.196	0.189	0.179
62.5	0.408 0.418	0.413	0.403
125	0.742 0.784	0.763	0.753
250	1.414 1.523	1.469	1.459
500	2.487 2.520	2.504	2.494

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 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	28	22	27
Mean (pg/mL)	30	87	232	29	85	238
Standard deviation	2.0	3.4	8.1	1.8	7.5	18.1
CV (%)	6.7	3.9	3.5	6.2	8.8	7.6

RECOVERY

The recovery of mouse 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=5)	110	86-120%
Serum (n=5)	99	84-113%
Heparin plasma (n=6)	101	81-115%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-6 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=5)	Serum (n=4)	Heparin plasma (n=5)
1:2	Average % of Expected	99	98	102
	Range (%)	90-104	96-102	92-111
1:4	Average % of Expected	94	91	104
	Range (%)	86-106	89-94	96-115
1:8	Average % of Expected	94	88	99
	Range (%)	82-108	86-89	89-118
1:16	Average % of Expected	99	89	102
	Range (%)	83-120	83-97	93-119

SENSITIVITY

Six assays were evaluated and the minimum detectable dose (MDD) of mouse IL-6 ranged from 1.3-1.8 pg/mL. The mean MDD was 1.6 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 mouse IL-6 produced at R&D Systems®.

The NIBSC non-WHO Reference Material mouse IL-6 preparation 93/730 which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 100 ng of recombinant mouse IL-6, and was assigned an arbitrary unitage of 10,000 Units/ampule.

NIBSC/WHO 93/730: 1 Unit of standard = approximately 1.03 pg Quantikine™ Mouse IL-6

SAMPLE VALUES

Serum - Twenty individual mouse serum samples were evaluated for the presence of mouse IL-6 in this assay. In all samples, IL-6 levels measured below the low standard, 7.8 pg/mL.

A mouse was injected with 15 µg of LPS and was bled 2 hours after injection. The serum was tested for mouse IL-6 and measured 31,800 pg/mL.

Heparin Plasma - Eight individual mouse heparin plasma samples were evaluated for the presence of mouse IL-6 in this assay. Six samples were below the low standard, 7.8 pg/mL. Two samples measured 23 pg/mL and 18 pg/mL.

Cell Culture Supernates:
EL-4 mouse lymphoblast cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL PHA and 10 µg/mL PMA. The culture supernate was assayed for mouse IL-6 and measured 386 pg/mL.

D10.G4.1 mouse helper T cells (1 x 10^5 cells/mL) were cultured in RPMI 1640 supplemented with L-glutamine, 10% fetal bovine serum, 50 µM β-mercaptoethanol, and 2.5% rat T cell polyclone media (Collaborative # 40115). During the final 3 days of culture, cells were stimulated with 2.5 ng/mL LPS. The culture supernate was assayed for mouse IL-6 and measured 759 pg/mL.

Mouse heart cells were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. The culture supernate was assayed for mouse IL-6 and measured 1120 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse 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 mouse IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
CT-1
gp130
IL-6 sR
IL-11
LIF
OSM

Recombinant human:
IL-6
IL-6 sR

Recombinant porcine:
IL-6

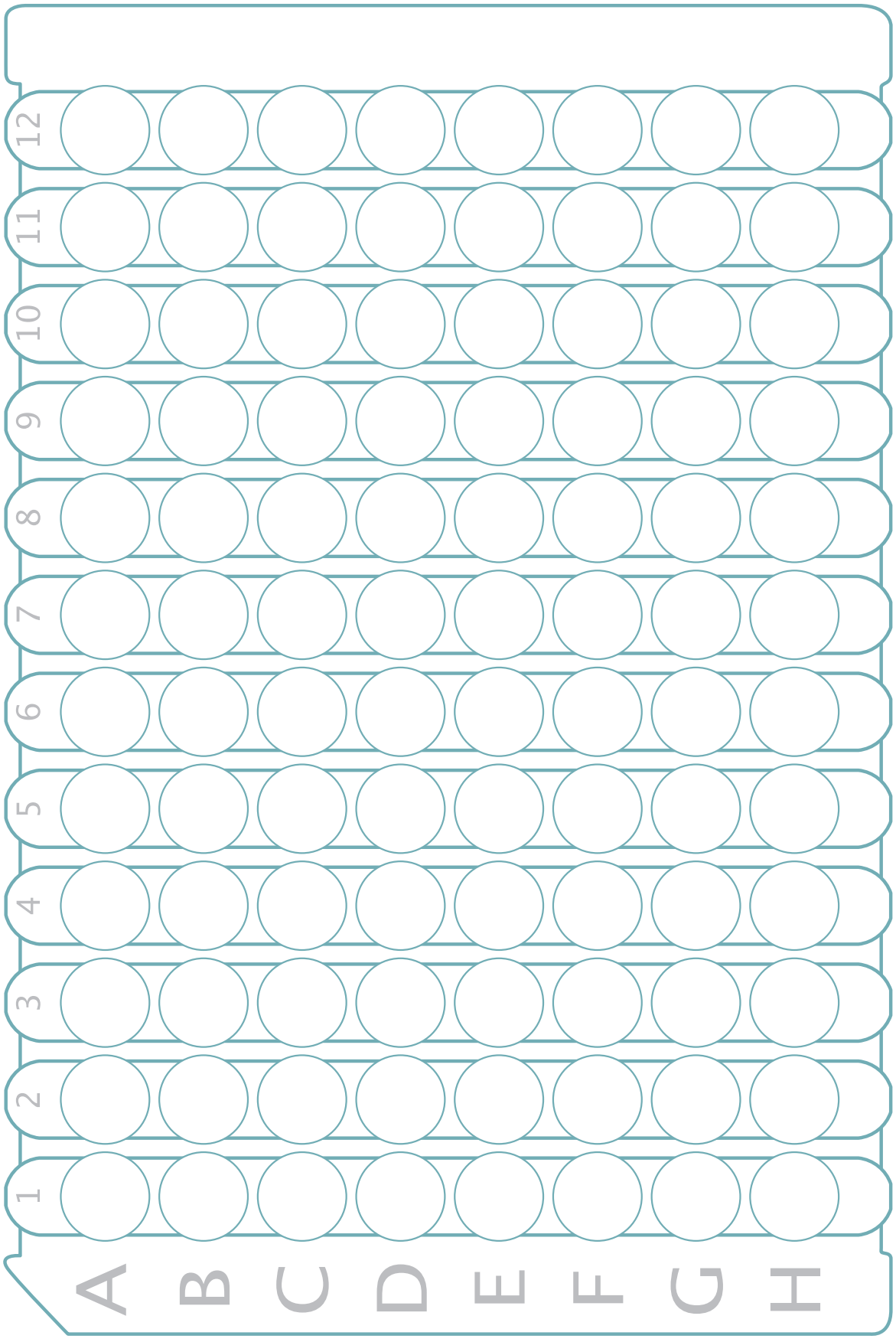
Recombinant rat IL-6 cross-reacts approximately 0.1% in this assay.

REFERENCES

1. Mansell, A. and B.J. Jenkins (2013) Cytokine Growth Factor Rev. **24**:249.
2. Schuett, H. *et al.* (2009) Thromb. Haemost. **102**:215.
3. Erta, M. *et al.* (2012) Int. J. Biol. Sci. **8**:1254.
4. Garbers, C. *et al.* (2012) Cytokine Growth Factor Rev. **23**:85.
5. Mihara, M. *et al.* (2012) Clin. Sci. (Lond.) **122**:143.
6. Chiu, C.P. *et al.* (1988) Proc. Natl. Acad. Sci. USA **85**:7099.
7. Simpson, R.J. *et al.* (1988) Eur. J. Biochem. **176**:187.
8. Van Snick, J. *et al.* (1988) Eur. J. Immunol. **18**:193.
9. May, L.T. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:8957.
10. Sad, S. *et al.* (1995) Immunity **2**:271.
11. Cichy, J. *et al.* (1996) Biochem. Biophys. Res. Commun. **227**:318.
12. Miyazawa, K. *et al.* (1998) Am. J. Pathol. **152**:793.
13. Fried, S.K. *et al.* (1998) Endocrinology **83**:847.
14. Ishimi, Y. *et al.* (1990) J. Immunol. **145**:3297.
15. Jiang, S. *et al.* (1994) Blood **84**:4151.
16. Xin, X. *et al.* (1995) Endocrinology **136**:132.
17. Marz, P. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:3251.
18. Ringheim, G.E. *et al.* (1995) J. Neuroimmunol. **63**:113.
19. Gadient, R.A. *et al.* (1995) Neurosci. Lett. **194**:17.
20. Kuppner, M.C. *et al.* (1995) Immunology **84**:265.
21. Gagari, E. *et al.* (1997) Blood **89**:2654.
22. Cumberbatch, M. *et al.* (1996) Immunology **87**:513.
23. Fujisawa, H. *et al.* (1997) J. Interferon Cytokine Res. **17**:347.
24. Lee, S.C. *et al.* (1993) J. Immunol. **150**:2659.
25. Lafortune, L. *et al.* (1996) J. Neuropathol. Exp. Neurol. **55**:515.
26. Ericson, S.G. *et al.* (1998) Blood **91**:2099.
27. Melani, C. *et al.* (1993) Blood **81**:2744.
28. Lacy, P. *et al.* (1998) Blood **91**:2508.
29. Jung, H.C. *et al.* (1995) J. Clin. Invest. **95**:55.
30. Spencer, N.F.L. and R.A. Daynes (1997) Int. Immunol. **9**:745.
31. Campbell, I.L. *et al.* (1989) J. Immunol. **143**:1188.
32. D'Auria, L. *et al.* (1997) Eur. Cytokine Netw. **8**:383.
33. Yamamura, M. *et al.* (1998) Br. J. Haematol. **100**:129.
34. Angstwurm, M.W.A. *et al.* (1997) Cytokine **9**:370.
35. Mouawad, R. *et al.* (1996) Clin. Cancer Res. **2**:1405.
36. Sakamoto, K. *et al.* (1994) Cytokine **6**:181.
37. Murakami, M. *et al.* (1993) Science **260**:1808.
38. Muller-Newen, G. (2003) Sci. STKE **2003**:PE40.
39. Mitsuyama, K. *et al.* (2006) Clin. Exp. Immunol. **143**:125.
40. Cerutti, A. *et al.* (1998) J. Immunol. **160**:2145.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES