Quantikine®

Rat IL-10 Immunoassay

Catalog Number R1000

For the quantitative determination of rat interleukin 10 (IL-10) 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

MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place N.E.
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 379-6580
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe
19 Barton Lane
Abingdon Science Park
Abingdon, Oxon OX14 3NB
United Kingdom

TELEPHONE: (0)1235 529449
FAX: (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems GmbH
Borsigstrasse 7
5205 Wiesbaden-Nordenstadt
Germany

FREEPHONE: (0)800 909 4455
TELEPHONE: (0)6122 90980
FAX: (0)6122 909819
E-MAIL: infogmbh@RnDSystems.co.uk

R&D Systems Europe
77 boulevard Vauban
59800 Lille
France

FREEPHONE: (0)800 90 72 49
FAX: (0)800 77 16 68
E-MAIL: info@RnDSystems.co.uk

INTRODUCTION

Interleukin 10 (IL-10), originally known as cytokine synthesis inhibitory factor (CSIF), is a pleiotropic cytokine with a myriad of immunomodulatory effects on a variety of cell types (1 - 5). On activated B cells, IL-10 can induce the formation of plasma cells (6) and the secretion of IgG (7, 8) or, in the presence of TGF-β1 and/or IL-4, the secretion of IgA (8, 9). On macrophages, IL-10 is known to downregulate IL-1, TNF-α and IL-6 production (10). On dendritic cells, IL-10 has been shown to interfere with antigen-presenting cell (APC) functions by downmodulating the expression of stimulatory and co-stimulatory molecules (11, 12). IL-10 has also been found to inhibit dendritic cell differentiation from monocytes and to promote monocyte maturation into cytotoxic $CD16^+$ macrophages (13 - 15). A number of epithelial and mesodermal cell types, including NK cells (16), cytotoxic ($CD8^+$) T cells secreting Th2 cytokines (17), $CD4^+CD45RA^-$ (memory) Th1 and Th2 cells (18), macrophages (19), monocytes (20), $CD5^+$ and $CD5^-$ B cells (21, 22), dendritic cells (Peyer's Patch-derived) (23), hepatic stellate (Ito) cells (24), keratinocytes (25), melanoma cells (26) and bronchogenic carcinoma cells (27), have been shown to produce IL-10.

Rat IL-10 cDNA encodes a 178 amino acid (aa) residue precursor protein that contains an 18 aa signal sequence and a 160 aa mature protein (1, 2). Within the mature protein, there are two potential N-linked glycosylation sites and five cysteine residues. Four of the five cysteine residues are involved in the formation of two intrachain disulfide linkages that are essential for activity (28). Mature rat IL-10 shares 85% and 74% aa sequence identity with mouse and human IL-10, respectively (1, 4). Based on human and murine studies, biologically active rat interleukin-10 is likely to be a nondisulfide-linked homodimer in solution (4, 29). Although IL-10 is considered to be a strictly secreted cytokine, the existence of biologically-active, membrane-bound IL-10 has also been reported. The exact mechanism by which IL-10 becomes membrane-associated is currently not known (30 - 32). Although rat and human IL-10 are both active on mouse cells (1, 33), mouse IL-10 is not active on human cells (4).

Based on human and mouse studies, the specific cell surface IL-10 signaling receptor complex is composed of two alpha (IL-10 R1) and two beta (IL-10 R2) chains (34 - 38). Both IL-10 R1 and IL-10 R2 are structurally related to the IFN-γ receptor, a member of the class II subgroup of the cytokine receptor family. Human IL-10 R1 cDNA encodes a 578 aa residue protein with a 21 aa signal sequence, a 215 extracellular domain, a 25 aa transmembrane domain and a 317 aa intracellular domain. IL-10 R1 has been shown to bind IL-10 with high-affinity (34, 35). IL-10 R2 is an accessory chain with broad tissue distribution and contains an approximately 100 aa cytoplasmic domain and a 200 aa extracellular domain (36). Cells known to express functional IL-10 receptor complexes include B cells, monocytes, and NK cells (35), keratinocytes (39), fibroblasts (40), Th1 T cells (40), and intestinal epithelial cells (41).

Bioassays for rat IL-10, using the cytokine synthesis inhibition assay or mast cell proliferation assay, MC/9, are time-consuming and not completely specific for rat IL-10. The Quantikine rat IL-10 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-10 levels in cell culture supernates, serum and plasma. It contains *E. coli*-expressed, recombinant rat IL-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant rat IL-10 accurately. Results obtained using natural rat IL-10 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that the Quantikine rat IL-10 Immunoassay kit can be used to determine relative mass values for natural rat IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IL-10 has been pre-coated onto a microplate. Standards, Controls, and samples are pipetted into the wells and any rat IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat 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 rat IL-10 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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all receptors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.

REAGENTS

Rat IL-10 Microplate (Part 890741) - One 96 well microplate (12 strips of 8 wells) coated with monoclonal antibody specific for rat IL-10.

Rat IL-10 Conjugate Concentrate (Part 890743) - 0.65 mL of a 23-fold concentrated solution containing antibody against rat IL-10 conjugated to horseradish peroxidase, with preservatives.

Type 11 Conjugate Diluent (Part 895509) - 12.5 mL of diluent for diluting the conjugate concentrate, with preservatives.

Rat IL-10 Standard (Part 890742) - 3 vials (4 ng/vial) of recombinant rat IL-10 in a buffered protein base, with preservatives, lyophilized.

Rat IL-10 Control (Part 890744) - 3 vials of recombinant rat IL-10 in a buffered protein base with preservatives, lyophilized. The expected range of the rat IL-10 control is shown on the vial label.

Assay Diluent RD1-21 (Part 895215) - 12.5 mL of a buffered protein solution, with preservatives.

Calibrator Diluent RD5-3 (Part 895436) - 21 mL of a buffered protein solution, with preservatives. *For cell culture supernate samples*.

Calibrator Diluent RD5T (Part 895175) - 21 mL of a buffered protein solution, with preservatives. *For serum/plasma samples*.

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant, with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid solution.

Plate Covers (Part 640197) - 4 adhesive plate sealers.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use beyond kit expiration date.</td></tr>
<tr><td rowspan="14">Opened/ Reconstituted Reagents</td><td>Diluted rat IL-10 Conjugate</td><td>May be stored for up to 1 week at 2 - 8° C.*</td></tr>
<tr><td>Diluted Wash Buffer</td><td rowspan="10">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD5-3</td></tr>
<tr><td>Calibrator Diluent RD5T</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Conjugate Concentrate</td></tr>
<tr><td>Conjugate Diluent</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Rat IL-10 Standard (2000 pg/mL)</td><td rowspan="2">Discard within 8 hours of reconstitution. Use a new Standard and Control for each assay.</td></tr>
<tr><td>Rat IL-10 Control</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*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.
- Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer.
- 1000 mL graduated cylinder.
- **Polypropylene test tubes.**

SAMPLE COLLECTION AND STORAGE

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 or overnight at 2 - 8° C before centrifuging for 20 minutes at approximately 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 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: *Grossly hemolyzed or lipemic samples may not be suitable for measurement of rat IL-10 with this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Rat IL-10 Kit Control - Reconstitute control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

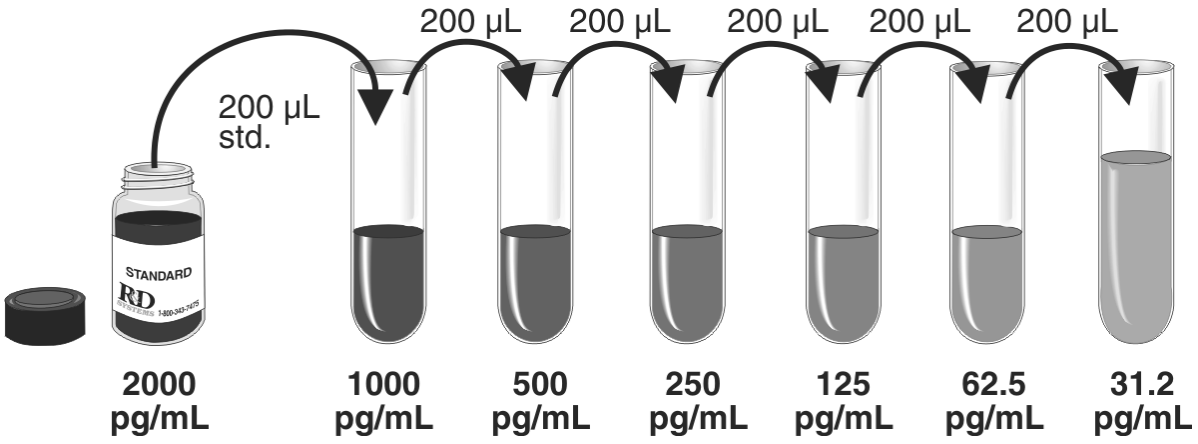
Rat IL-10 Conjugate - To prepare enough conjugate for the entire plate, add 0.5 mL Conjugate Concentrate to 11.0 mL Conjugate Diluent. Use a sterile container, and protect from light.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough wash buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 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.

Rat IL-10 Standard - Reconstitute the rat IL-10 Standard with 2.0 mL of Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or 2.0 mL Calibrator Diluent RD5T (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene test tubes. Pipette 200 µL of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted rat IL-10 Standard serves as the high standard (2000 pg/mL). The appropriate Calibrator Diluent 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 samples, standards and controls be assayed in duplicate.

1. Prepare reagents and standard dilutions 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, reseal.

3. Add 50 µL of Assay Diluent RD1-21 to each well.

4. Add 50 µL of Standard, Control or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. 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, multi-channel pipette, 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 rat 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.

PROCEDURE SUMMARY AND CHECKLIST

1. ☐ Bring all reagents to room temperature.
☐ Prepare reagents and samples as instructed.
☐ Return unused components to storage temperature as indicated in the instructions.

2. ☐ Add 50 µL Assay Diluent to the center of each well.

3. ☐ Add 50 µL Standard, Control, or sample to the center of each well.
☐ Tap plate gently for one minute.
☐ Cover the plate and incubate 2 hours at room temperature.

4. ☐ Aspirate and wash each well five times.

5. ☐ Add 100 µL Conjugate to each well.
☐ Cover the plate and incubate 2 hours at room temperature.

6. ☐ Aspirate and wash each well five times.

7. ☐ Add 100 µL Substrate Solution to each well. Incubate 30 minutes at room temperature. **Protect from light.**

8. ☐ Add 100 µL Stop Solution to each well.

9. ☐ Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

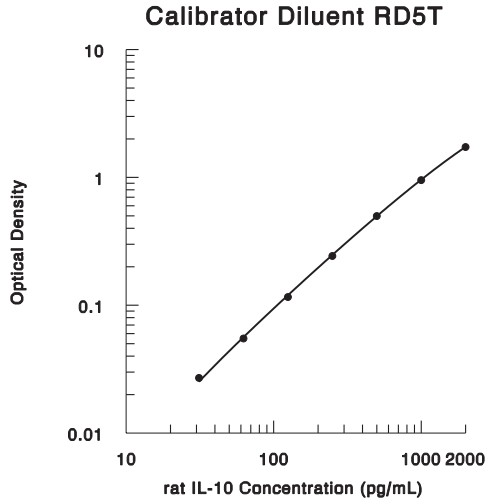
CALCULATION OF RESULTS

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

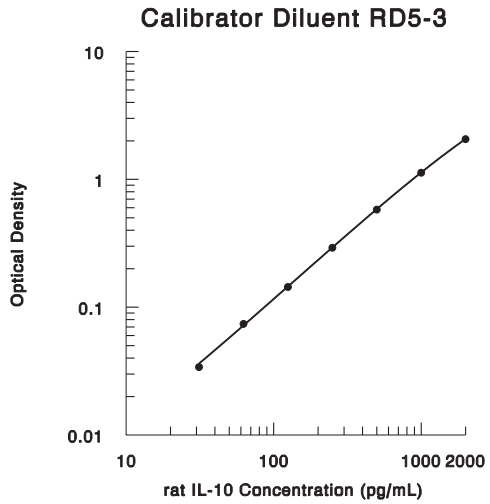
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the rat 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.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.049 0.053	0.051	——
31.2	0.078 0.078	0.078	0.027
62.5	0.107 0.105	0.106	0.055
125	0.169 0.165	0.167	0.116
250	0.295 0.293	0.294	0.243
500	0.553 0.546	0.550	0.499
1000	0.994 1.015	1.004	0.953
2000	1.767 1.798	1.782	1.731



(pg/mL)	O.D.	Average	Corrected
0	0.067 0.065	0.066	——
31.2	0.099 0.100	0.100	0.034
62.5	0.139 0.141	0.140	0.074
125	0.211 0.208	0.210	0.144
250	0.357 0.358	0.358	0.292
500	0.654 0.637	0.646	0.580
1000	1.194 1.194	1.194	1.128
2000	2.055 2.210	2.132	2.066

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 assays to assess inter-assay precision.

Serum/Plasma Assay

	Intra-assay Precision			Inter-assay precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	138	270	660	132	270	682
Standard deviation	6.3	9.0	16.1	13.1	19.7	59.8
CV (%)	4.6	3.3	2.4	9.9	7.3	8.8

Cell Culture Supernate Assay

	Intra-assay Precision			Inter-assay precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	103	218	582	114	236	598
Standard deviation	5.7	9.5	17.2	10.8	16.4	42.6
CV (%)	5.5	4.4	3.0	9.5	6.9	7.1

RECOVERY

The recovery of rat IL-10 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)	106	96 - 118%
Rat serum (n = 6)	94	82 - 112%
Rat EDTA plasma (n = 6)	100	88 - 115%

LINEARITY

To assess the linearity of the assay, five or more samples spiked with various concentrations of rat IL-10 in each matrix were diluted with the appropriate Calibrator Diluent and then assayed. Results from typical sample dilutions are shown.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell Culture Supernates	spiked	1378		
	1/2	697	689	101%
	1/4	342	344	99%
	1/8	166	172	97%
	1/16	81	86	94%
Rat Serum	spiked	1034		
	1/2	506	517	98%
	1/4	267	258	103%
	1/8	131	129	102%
	1/16	69	64	108%
Rat EDTA Plasma	spiked	1177		
	1/2	616	588	105%
	1/4	312	294	106%
	1/8	147	147	100%
	1/16	70	74	95%

SENSITIVITY

The minimum detectable dose of rat IL-10 is typically less than 10 pg/mL.

The minimum detectable dose 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 highly purified, *E. coli*-expressed, recombinant rat IL-10 produced at R&D Systems. This recombinant form of rat IL-10 contains 160 amino acid residues and has a predicted molecular mass of 19 kDa.

Based on total amino acid analysis, the absorbance of a 1 mg/mL solution of the *E. coli*-expressed, recombinant rat IL-10 at 280 nm was determined to be 1.15 A.U.

SAMPLE VALUES

Serum - Twenty individual rat serum samples were evaluated for detectable levels of rat IL-10 in this assay. Nineteen samples measured below the lowest standard, 31.2 pg/mL. One sample measured 34.8 pg/mL.

Plasma - Twenty individual rat EDTA plasma samples were evaluated for detectable levels of rat IL-10 in this assay. Fourteen samples measured below the lowest standard, 31.2 pg/mL. Six samples measured from 32.3 to 59.9 pg/mL.

Cell Culture Supernates - Rat splenocytes (1 x 10^7 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal calf serum and stimulated with 5 μg/mL Conconavalin A. The culture supernate was assayed for rat IL-10 and measured 340 pg/mL.

SPECIFICITY

This assay recognizes both recombinant and natural rat IL-10. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and RD5T, and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range rat IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:
CINC-1
GDNF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-18
β-NGF
PDGF-BB
TNF-α

Recombinant mouse:
IL-10 sR

Recombinant human:
IL-10
IL-10 sR

Some cross-reactivity was observed with the following:

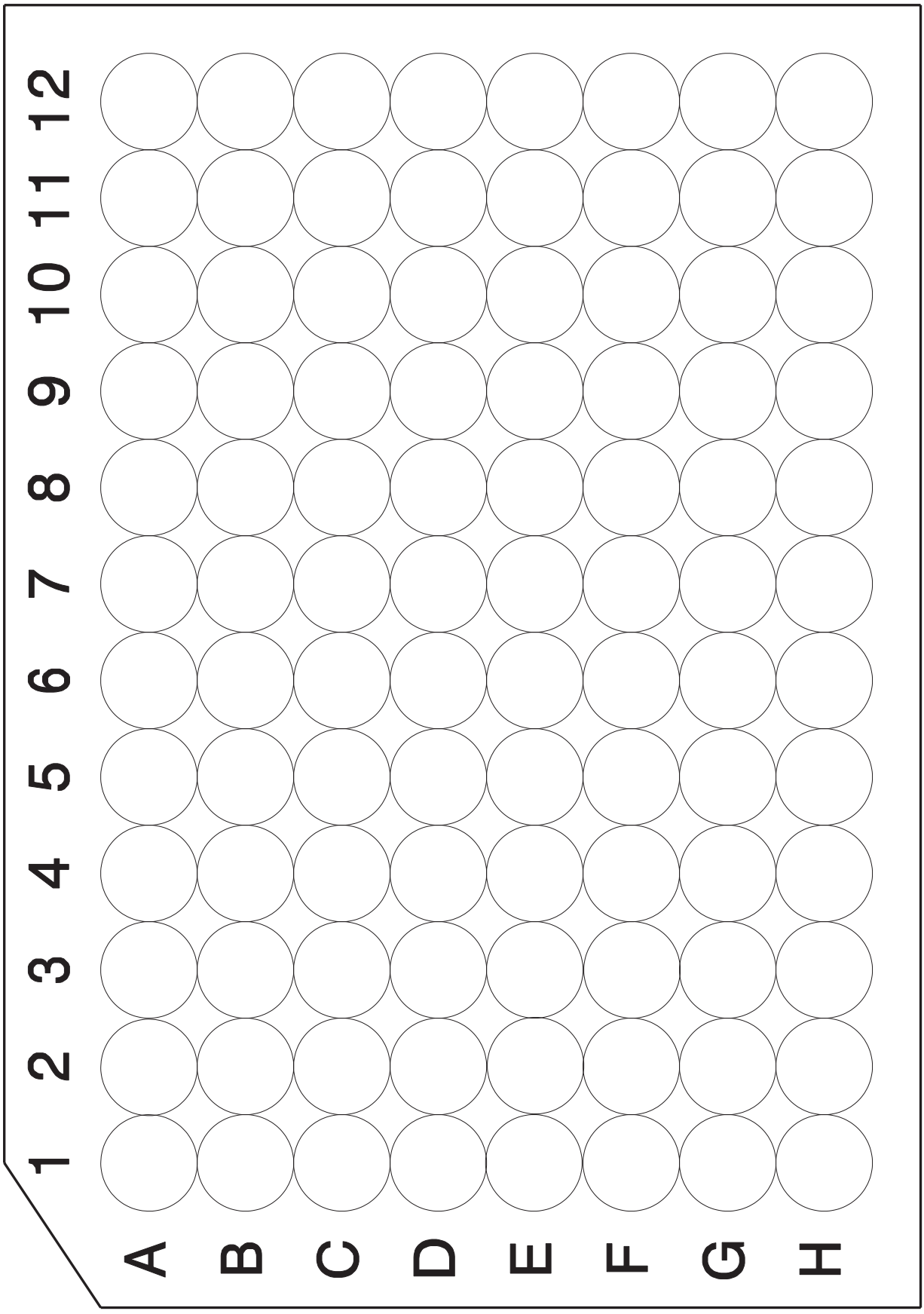
Recombinant Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
mouse IL-10	50,000	900	1.8

REFERENCES

1. Feng, L. *et al*. (1993) Biochem. Biophys. Res. Commun. **192**:452.
2. Goodman, R.E. *et al*. (1992) Biochem. Biophys. Res. Commun. **189**:1.
3. Fiorentino, D.F. *et al*. (1989) J. Exp. Med. **170**:2081.
4. Mosmann, T.R. (1994) Adv. Immunol. **56**:1.
5. Moore, K.W. *et al*. (1990) Science **248**:1230.
6. Rousset, F. *et al*. (1995) Int. Immunol. **7**:1243.
7. Briere, F. *et al*. (1994) J. Exp. Med. **179**:757.
8. Defrance, T. *et al*. (1992) J. Exp. Med. **175**:671.
9. Marconi, M. *et al*. (1998) Clin. Exp. Immunol. **112**:528.
10. Fiorentino, D.F. *et al*. (1991) J. Immunol. **147**:3815.
11. Qzawa, H. *et al*. (1996) Eur. J. Immunol. **26**:648.
12. Buelens, C. *et al*. (1995) Eur. J. Immunol. **25**:2668.
13. Olikowsky, T. *et al*. (1997) Immunology **91**:104.
14. Calzada-Wack, J.C. *et al*. (1996) J. Inflamm. **46**:78.
15. Allavena, P. *et al*. (1998) Eur. J. Immunol. **28**:359.
16. Mehrotra, P.T. *et al*. (1998) J. Immunol. **160**:2637.
17. Sad, S. *et al*. (1995) Immunity **2**:271.
18. Yssel, H. *et al*. (1992) J. Immunol. **149**:2378.
19. Panuska, J.R. *et al*. (1995) J. Clin. Invest. **96**:2445.
20. Hodge, S. *et al*. (1999) Scand. J. Immunol. **49**:548.
21. Spencer, N.F.L. and R.A. Daynes (1997) Int. Immunol. **9**:745.
22. O'Garra, A. *et al*. (1990) Int. Immunol. **2**:821.
23. Iwasaki, A. and B.L. Kelsall (1999) J. Exp. Med. **190**:229.
24. Wang, S.C. *et al*. (1998) J. Biol. Chem. **273**:302.
25. Grewe, M. *et al*. (1995) J. Invest. Dermatol. **104**:3.
26. Sato, T. *et al*. (1996) Clin. Cancer Res. **2**:1383.
27. Smith, D.R. *et al*. (1994) Am. J. Pathol. **145**:18.
28. Windsor, W.T. *et al*. (1993) Biochemistry **32**:8807.
29. Syto, R. *et al*. (1998) Biochemistry **37**:16943.
30. Fleming, S.D. and P.A. Campbell (1996) J. Immmunol. **156**:1143.
31. Thompson, K.C. *et al*. (1998) Hepatology **28**:1518.
32. Capsoni, F. *et al*. (1998) Cell. Immunol. **189**:51.
33. Vieira, P. *et al*. (1991) Proc. Natl. Acad. Sci. USA **88**:1172.
34. Ho, A.S.Y. *et al*. (1993) Proc. Natl. Acad. Sci. USA **90**:11267.
35. Liu, Y. *et al*. (1994) J. Immunol. **152**:1821.
36. Gibbs, V.C. and D. Pennica (1997) Gene **186**:97.
37. Kotenko, S.V. *et al*. (1997) EMBO J. **16**:5894.
38. Donnelly, R.P. *et al*. (1999) J. Interferon Cytokine Res. **19**:563.
39. Michel, G. *et al*. (1997) J. Immunol. **159**:6291.
40. Weber-Nordt, R.M. *et al*. (1994) J. Immunol. **153**:3734.
41. Song, F. *et al*. (1999) J. Immunol. **162**:2275.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES