Quantikine® ELISA

Porcine IL-10 Immunoassay

Catalog Number P1000

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

Note: The standard reconstitution method has changed. Please 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.

TABLE OF CONTENTS

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 10, also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 cytokine family. This family currently comprises IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-4). All IL-10 family members are secreted α-helical proteins. Porcine IL-10 is a secreted, possibly glycosylated, polypeptide with an 18 kDa molecular weight (5). Based on human studies, porcine IL-10 is likely to circulate as a nondisulfide-linked homodimer (3). Porcine IL-10 is synthesized as a 175 amino acid (aa) precursor with an 18 aa signal sequence and a 157 aa mature form. The mature segment has one potential N-linked glycosylation site plus four cysteines which form two intrachain disulfide bridges (6). Mature porcine IL-10 shows 71%, 70%, 76%, 75%, 77%, and 71% aa sequence identity to rat (7), mouse (8), human (9), guinea pig (10), canine (11), and cotton rat (12) IL-10, respectively. Upon activation, mammalian cells known to secrete IL-10 include NK cells (13), cytotoxic $CD8^+$ T cells secreting Th2-like cytokines (14), $CD4^+CD45RA^-$ (memory) Th1 and Th2 cells (15), macrophages (16), monocytes (17), $CD5^+$ and $CD5^-$ B cells (18, 19), dendritic cells (20, 21), hepatic stellate (Ito cells) (22), keratinocytes (23), melanoma cells (24), mast cells (25), placental cytotrophoblasts (26), and fetal erythroblasts (27).

The functional receptor for IL-10 (IL-10 R) in pigs has not been reported. By analogy to human, it would be expected to be composed of two 110 kDa α-chains (or IL-10 R1) and two 75 kDa β-chains (or IL-10 R2) (28-31). The α-chain binds IL-10 and transduces a signal in the presence of a β-chain complex (29). Both receptors are members of the class II cytokine receptor family (CRF2) that is characterized by the presence of type III fibronectin domains and conserved tryptophans (29, 31). This class does not possess the WSXWS motif characteristic of the class I CRF. There is no significant aa sequence identity (< 30%) between human IL-10 R1 and IL-10 R2.

IL-10 has myriad effects on a variety of cell types. On activated B cells, IL-10 can induce plasma cell formation (32) and the secretion of either IgG (33, 34) or IgA (in the presence of TGF-β1 and/or IL-4) (34, 35). In the presence of IL-2, $CD56^+$ NK cells will respond to IL-10 with increased proliferation plus IFN-γ and TNF-α secretion (36). Conversely, on macrophages, IL-10 is known to downregulate IL-1, TNF-α, and IL-6 production (37). On dendritic cells, IL-10 has been shown to interfere with antigen-presenting cell function by downmodulating stimulatory and co-stimulatory molecules (38, 39). On monocytes, IL-10 is reported to direct monocyte differentiation into cytotoxic $CD16^+$ macrophages rather than antigen-presenting dendritic cells (40-42).

The Quantikine Porcine IL-10 Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-10 levels in cell culture supernates, serum, and EDTA plasma. It contains *E. coli*-expressed recombinant porcine IL-10 and antibodies raised against the recombinant protein. Results obtained for naturally occurring porcine 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 of natural porcine IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for porcine IL-10 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any porcine IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for porcine 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 porcine 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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Porcine IL-10 Microplate	892294	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for porcine 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.*
Porcine IL-10 Standard	892296	2 vials of recombinant porcine 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.
Porcine IL-10 Control	892297	2 vials of recombinant porcine 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.	
Porcine IL-10 Conjugate	892295	23 mL of a polyclonal antibody specific for porcine IL-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-33	895349	21 mL of a buffered protein base 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.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

Calibrator Diluent RD6-33 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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. 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 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 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: *Heparin and citrate plasma have not been validated for use in this assay.*

SAMPLE PREPARATION

Cell culture supernate, serum, and EDTA plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 120 µL of sample + 120 µL of Calibrator Diluent RD6-33.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

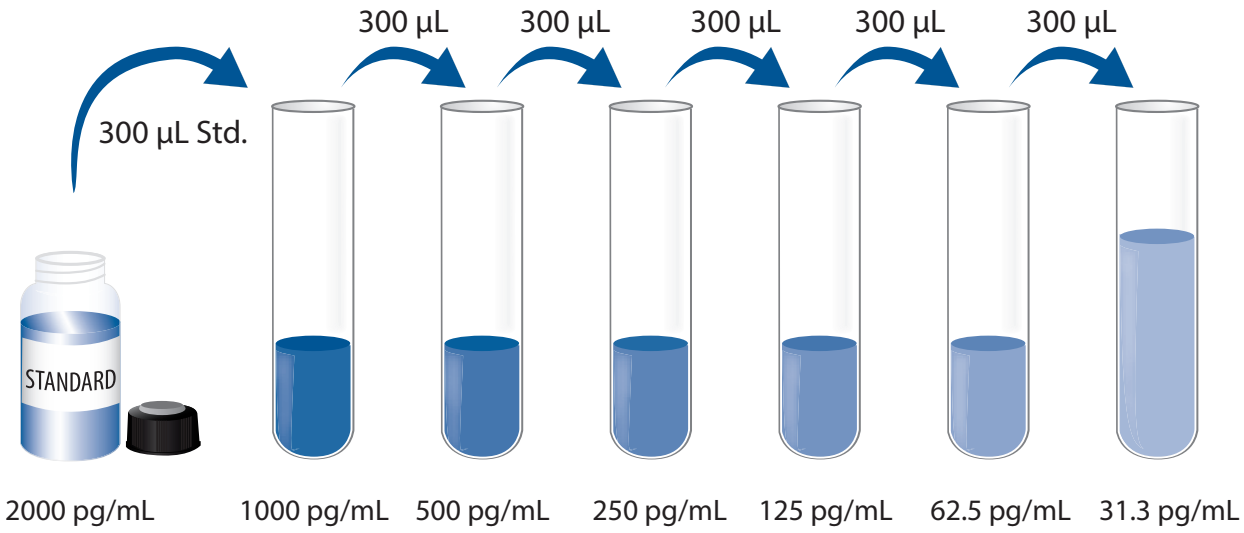
Porcine IL-10 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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. 120 µL of the resultant mixture is required per well.

Porcine IL-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Porcine IL-10 Standard with Calibrator Diluent RD6-33. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 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 300 µL of Calibrator Diluent RD6-33 into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Porcine IL-10 Standard(2000 pg/mL) serves as the high standard. Calibrator Diluent RD6-33 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, 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 100 µL of Assay Diluent RD1W to each well.
4. Add 100 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.
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 200 µL of Porcine IL-10 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 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 120 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

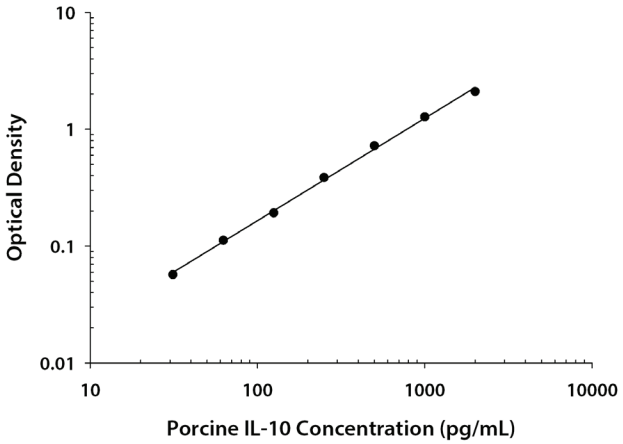
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the porcine IL-10 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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	0.068 0.074	0.071	—
31.3	0.127 0.128	0.128	0.057
62.5	0.179 0.187	0.183	0.112
125	0.262 0.263	0.263	0.192
250	0.449 0.465	0.457	0.386
500	0.788 0.798	0.793	0.722
1000	1.335 1.359	1.347	1.276
2000	2.167 2.179	2.173	2.102

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	88	264	791	89	264	802
Standard deviation	3.7	6.8	27.5	6.4	11.8	54.7
CV (%)	4.2	2.6	3.5	7.2	4.5	6.8

RECOVERY

The recovery of porcine 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 supernates (n=4)	106	101-109%
Serum (n=4)	94	84-102%
EDTA plasma (n=4)	92	80-100%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of porcine 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 supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	105	103	107
	Range (%)	103-106	100-106	104-109
1:4	Average % of Expected	99	99	102
	Range (%)	97-100	94-104	98-105
1:8	Average % of Expected	92	93	94
	Range (%)	90-94	85-98	86-98
1:16	Average % of Expected	87	91	92
	Range (%)	84-90	80-100	85-96

SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of porcine IL-10 ranged from 1.8-5.5 pg/mL. The mean MDD was 3.5 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-10 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma -Fifteen samples were evaluated for detectable levels of porcine IL-10 in this assay. One EDTA plasma sample measured 118 pg/mL, while all remaining samples measured below the lowest standard, 31.3 pg/mL.

Cell Culture Supernates - Porcine peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum and stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin for 20 hours. An aliquot of the cell culture supernate was removed, assayed for porcine IL-10, and measured 254 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant porcine 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 the same concentrations in a mid-range porcine IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:

GM-CSF
IFN-γ
IL-2
IL-4
IL-6
IL-8
TNF-α

Recombinant rat:

IFN-γ
IL-4
IL-10

Recombinant mouse:

IFN-γ
IL-4
IL-10
IL-10 R

Recombinant human:

IFN-γ
IL-4
IL-10
IL-10 R

Other recombinants:

canine IFN-γ
canine IL-4
cotton rat IL-10
feline IL-10

Natural proteins:

human PDGF
viral IL-10 (human cytomegalovirus)
viral IL-10 (Epstein-Barr virus)

Recombinant canine IL-10 cross-reacts at concentrations ≥ 12.5 ng/mL.

REFERENCES

1. Rich, B.E. and T.S. Kupper (2001) Curr. Biol. **11**:R531.
2. Gruenberg, B.H. *et al.* (2001) Genes Immun. **2**:329.
3. Moore, K.W. *et al.* (2001) Annu. Rev. Immunol. **19**:683.
4. Fickenscher, H. et. al. (2002) Trends Immunol. **23**:89.
5. Blancho, G. *et al.* (1995) Proc. Natl. Acad. Sci. USA **92**:2800.
6. Windsor, W.T. *et al.* (1993) Biochemistry **32**:8807.
7. Feng, L. *et al.* (1993) Biochem. Biophys. Res. Commun. **192**:452.
8. Moore, K.W. *et al.* (1990) Science **248**:1230.
9. Vieira, P. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:1172.
10. Scarozza, A.M. *et al.* (1998) Cytokine **10**:851.
11. Lu, P. *et al.* (1995) J. Interf. Cytokine Res. **15**:1103.
12. Langley, R.J. *et al.* (2001) GenBank Accession #AAK04013.
13. Mehrotra, P.T. *et al.* (1998) J. Immunol. **160**:2637.
14. Sad, S. *et al.* (1995) Immunity **2**:271.
15. Yssel, H. *et al.* (1992) J. Immunol. **149**:2378.
16. Panuska, J.R. *et al.* (1995) J. Clin. Invest. **96**:2445.
17. Hodge, S. *et al.* (1999) Scand. J. Immunol. **49**:548.
18. Spencer, N.F.L. and R.A. Daynes (1997) Int. Immunol. **9**:745.
19. O'Garra, A. *et al.* (1990) Int. Immunol. **2**:821.
20. Iwasaki, A. and B.L. Kelsall (1999) J. Exp. Med. **190**:229.
21. Rea, D. *et al.* (2000) Blood **95**:3162.
22. Wang, S.C. *et al.* (1998) J. Biol. Chem. **273**:302.
23. Grewe, M. *et al.* (1995) J. Invest. Dermatol. **104**:3.
24. Sato, T. *et al.* (1996) Clin. Cancer Res. **2**:1383.
25. Ishizuka, T. *et al.* (1999) Clin. Exp. Allergy **29**:1424.
26. Roth, I. *et al.* (1996) J. Exp. Med. **184**:539.
27. Sennikov, S.V. *et al.* (2002) Cytokine **17**:221.
28. Kotenko, S.V. *et al.* (1997) EMBO J. **16**:5894.
29. Lutfalla, G. *et al.* (1993) Genomics **16**:366.
30. Gibbs, V.C. and D. Pennica (1997) Gene **186**:97.
31. Liu, Y. *et al.* (1994) J. Immunol. **152**:1821.
32. Rousset, F. *et al.* (1995) Int. Immunol. **7**:1243.
33. Briere, F. *et al.* (1994) J. Exp. Med. **179**:757.
34. Defrance, T. *et al.* (1992) J. Exp. Med. **175**:671.
35. Marconi, M. *et al.* (1998) Clin. Exp. Immunol. **112**:528.
36. Carson, W.E. *et al.* (1995) Blood **85**:3577.
37. Fiorentino, D.F. *et al.* (1991) J. Immunol. **147**:3815.
38. Qzawa, H. *et al.* (1996) Eur. J. Immunol. **26**:648.
39. Buelens, C. *et al.* (1995) Eur. J. Immunol. **25**:2668.
40. Olikowsky, T. *et al.* (1997) Immunology **91**:104.
41. Calzada-Wack, J.C. *et al.* (1996) J. Inflamm. **46**:78.
42. Allavena, P. *et al.* (1998) Eur. J. Immunol. **28**:359.