Quantikine®

Human IL-6 Immunoassay

Catalog Number D6050

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

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Interleukin 6 (IL-6) is a multifunctional protein produced by lymphoid and non-lymphoid cells, and by normal and transformed cells, including T cells, monocyte/macrophages, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astrogliomas and glioblastomas. The production of IL-6 in these various cells is regulated, either positively or negatively, by a variety of signals including mitogens, antigenic stimulation, lipopolysaccharides, IL-1, TNF, PDGF and viruses. On the basis of its various activities, IL-6 has also been called interferon-β2 (IFN-β2), 26-kDa protein, B-cell stimulatory factor-2 (BSF-2), hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, cytotoxic T-cell differentiation factor, and macrophage-granulocyte inducing factor 2A (MGI-2A). For reviews on IL-6, see references 1-5.

The human IL-6 cDNA sequence predicts a protein of 212 amino acid residues in length with two potential N-glycosylation sites. The hydrophobic N-terminal 28 amino acid residue signal peptide is cleaved to produce a mature protein of 184 amino acids with four cysteine residues and a predicted molecular mass of 21 kDa (6 - 9). Mouse IL-6 cDNA sequence shows a homology of 42% at the amino acid level when compared with the human sequence (10). Sequencing of the genomic DNA for IL-6 indicates that the gene for this factor consists of five exons and four introns. On the basis of sequence similarity and gene structural motif similarity, IL-6 can be grouped in a family of cytokines that also includes OSM, G-CSF, LIF, and CNTF. All of these cytokines are predicted to have a four helix bundle structure similar to that found for growth hormone, suggesting that they all evolved from a common ancestral gene (11, 38, 39).

The effects of IL-6 on different cells are numerous and varied. The effect on B cells is stimulation of differentiation and antibody secretion (6, 12 - 15). IL-6 also affects T cells, acting as a co-stimulant with sub-optimal concentrations of PHA or Con A to stimulate IL-2 production and IL-2 receptor expression. IL-6 exhibits growth factor activity for mature thymic or peripheral T-cells and reportedly enhances the differentiation of cytotoxic T-cells in the presence of IL-2 or IFN-γ (16 - 18). It stimulates production of acute phase proteins by hepatocytes (19) and has colony-stimulating activity on hematopoietic stem cells (20, 21). IL-6 has growth factor activities and will stimulate the growth of myeloma/hybridoma/plasmacytoma cells (22, 23), EBV-transformed B cells (24), keratinocytes and mesangial cells (4, 5). Additional bioactivities attributed to IL-6 include: inhibition of the growth and induction of terminal differentiation of M1 myeloid leukemic cells (25); induction of neuronal cell differentiation (26, 27); induction of the maturation of megakaryocytes (28). Although IL-6 was also discovered as an antiviral activity produced by human diploid fibroblasts, the question of whether or not IL-6 has antiviral activity is controversial. Many groups have been consistently unable to find any antiviral activity for recombinant human IL-6.

The various activities of IL-6 described above suggest that this factor will have a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. Although the exact functions of IL-6 *in vivo* are not known, elevated IL-6 levels have been reported to be associated with a variety of diseases, including autoimmune diseases such as arthritis and Castleman's disease (4, 5, 29, 30), mesangial proliferative glomerulonephritis (4, 5), psoriasis (31), inflammatory bowel disease (32), and malignancies such as plasmacytomas (4), myelomas (2, 3, 4, 33), lymphomas and leukemias (4, 34), and ovarian cancers (35). A great deal of work is in progress in order to provide a better understanding of the role of IL-6 in the modulation of normal and pathological processes (2, 5).

Current methods for assay of this interleukin are based on the mitogenic effects of IL-6 on appropriate cell lines, such as B9, a mouse B cell hybridoma line, or T1165.85.2.1, a mouse plasmacytoma cell line. These bioassays are time-consuming and are not completely specific for IL-6. The Quantikine IL-6 Immunoassay is a 4.5 hour solid phase ELISA designed to measure IL-6 in cell culture supernates, serum, and plasma. It contains recombinant human IL-6 and antibodies raised against recombinant human IL-6 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural IL-6 showed linear curves that were parallel to the standard curves obtained using the *E. coli*-expressed Quantikine kit standards. These results indicate that the Quantikine Immunoassay kit can be used to determine relative mass values for natural IL-6.

It has been observed in our laboratories that the measurement of IL-6 is insensitive to the addition of the recombinant form of the IL-6 soluble receptor. Therefore it is probable that experimental sample measurements reflect the total amount of IL-6 present, *i.e.*, the total amount of free IL-6 plus the amount of IL-6 initially bound to soluble receptors, if any are present in the samples. High levels of high-affinity autoantibodies to IL-6 in the serum of some normal blood donors have been reported (36, 37). Such autoantibodies have the potential to interfere with the measurement of IL-6 by ELISA immunoassays.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for 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 and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- 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. If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the appropriate Calibrator Diluent.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

REAGENTS

IL-6 Microplate (Part 890045) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against IL-6.

IL-6 Conjugate (Part 890046) - 21 mL of polyclonal antibody against IL-6 conjugated to horseradish peroxidase, with preservatives.

IL-6 Standard (Part 890047) - 1.5 ng of recombinant human IL-6 in a buffered protein base with preservatives, lyophilized.

Assay Diluent RD1A (Part 895005) - 11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD5A (Part 895010) - 21 mL of a buffered protein base with preservatives.
For cell culture supernate samples.

Calibrator Diluent RD6F (Part 895018) - 21 mL of animal serum with preservatives.
For serum/plasma samples.

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

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 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD5A</td></tr>
<tr><td>Calibrator Diluent RD6F</td></tr>
<tr><td>Assay Diluent RD1A</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at ≤ - 20° C. Avoid repeated freeze-thaw cycles.*</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.

PRECAUTIONS

Some components of this kit contain 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. Wear eye, hand, face, and clothing protection when using this material.

This kit contains Thimerosal, a mercury containing compound. The total amount of mercury in this kit is 2.55 mg.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 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, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

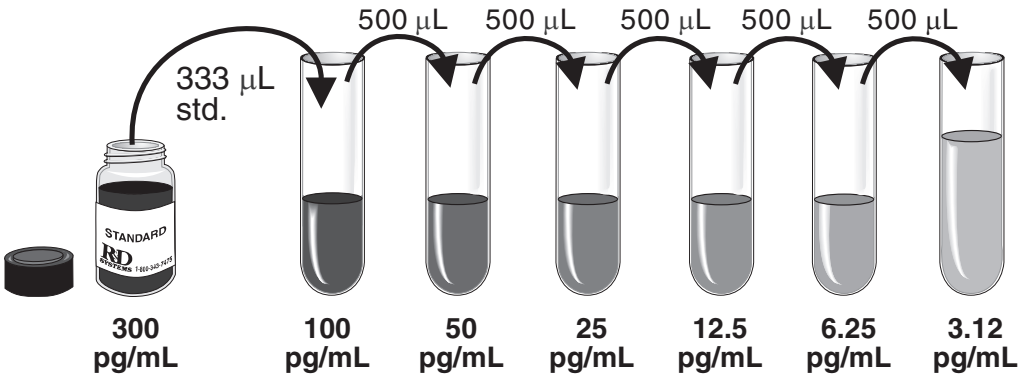
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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. 200 µL of the resultant mixture is required per well.

IL-6 Standard - Reconstitute the IL-6 Standard with 5 mL of Calibrator Diluent RD5A (for cell culture supernate samples) or Calibrator Diluent RD6F (for serum/plasma samples). This reconstitution produces a stock solution of 300 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 667 µL of the appropriate Calibrator Diluent into the 100 pg/mL tube and 500 µL of diluent into each remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (300 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 and standards be assayed in duplicate.

1. Prepare all reagents and working standards 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 100 µL of Assay Diluent RD1A to each well.

4. Add 100 µL of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.

5. Aspirate each well and wash, repeating the process three times for a total of four 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 toweling.

6. Add 200 µL of 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 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, 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.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents and standards as directed.

2. Add 100 µL Assay Diluent RD1A to each well.

3. Add 100 µL Standard or sample to each well. Incubate 2 hrs. RT

4. Aspirate and wash 4 times.

5. Add 200 µL Conjugate to each well. Incubate 2 hrs. RT

6. Aspirate and wash 4 times.

7. Add 200 µL Substrate Solution to each well. **Protect from light.** Incubate 20 min. RT

8. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 min. λ correction 540 or 570 nm

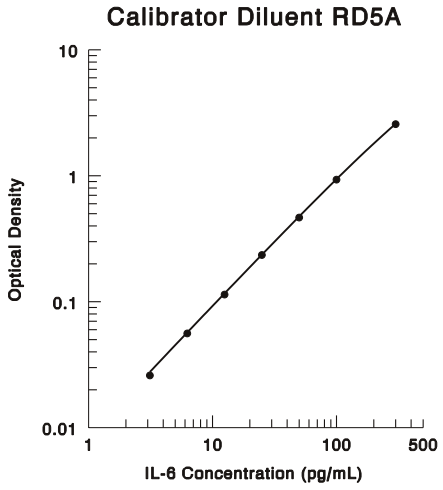
CALCULATION OF RESULTS

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

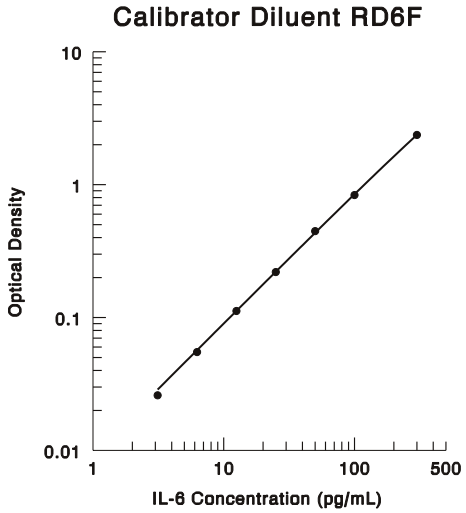
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

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	0.022 0.028	0.025	
3.12	0.050 0.052	0.051	0.026
6.25	0.078 0.078	0.078	0.053
12.5	0.134 0.136	0.135	0.110
25	0.247 0.245	0.246	0.221
50	0.472 0.465	0.468	0.443
100	0.865 0.836	0.850	0.825
300	2.524 2.515	2.520	2.495



(pg/mL)	O.D.	Average	Corrected
0	0.025 0.029	0.027	
3.12	0.049 0.051	0.050	0.023
6.25	0.078 0.077	0.078	0.051
12.5	0.127 0.129	0.128	0.101
25	0.236 0.236	0.236	0.209
50	0.438 0.442	0.440	0.413
100	0.780 0.773	0.776	0.749
300	2.176 2.221	2.198	2.171

TECHNICAL HINTS

- Substrate Solution should remain colorless until added to the plate. Keep the Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- 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.

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were assayed twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were assayed in twenty separate 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)	16.8	97.7	186	17.2	101	191
Standard deviation	0.7	1.6	3.8	1.1	3.3	7.2
CV (%)	4.2	1.6	2.0	6.4	3.3	3.8

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)	15.8	95.6	179	16.4	98.8	188
Standard deviation	0.7	3.0	3.1	0.6	2.5	3.7
CV (%)	4.4	3.1	1.7	3.7	2.5	2.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media	98	94 - 103%
Serum	93	86 - 99%
EDTA plasma	95	84 - 101%
Heparin plasma	90	88 - 98%
Citrate plasma	91	82 - 95%

LINEARITY

To assess the linearity of the assay, four samples were spiked with high concentrations of IL-6 in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media	Serum	EDTA plasma	Heparin plasma	Citrate plasma
1:2	Average % of Expected	99	97	101	103	101
	Range (%)	96-101	92-100	98-105	96-109	96-106
1:4	Average % of Expected	100	101	104	106	105
	Range (%)	93-110	93-107	97-110	97-113	101-109
1:8	Average % of Expected	96	102	100	104	106
	Range (%)	92-100	96-108	86-112	93-111	101-111
1:16	Average % of Expected	94	103	99	105	101
	Range (%)	83-108	93-111	90-110	99-107	90-114

SENSITIVITY

The minimum detectable dose of IL-6 is typically less than 0.70 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 a highly purified *E. coli*-expressed recombinant human IL-6 produced at R&D Systems. The NIBSC/WHO International Standard for IL-6 (89/548) (40), which was intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is a CHO cell-derived recombinant human IL-6.

The dose response curve of the International Standard (89/548) parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine IL-6 kit to equivalent NIBSC 89/548 units, use the equation below.

NIBSC (89/548) equivalent value (IU/mL) = 0.131 x Quantikine IL-6 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Forty serum and plasma samples were evaluated for the presence of IL-6 in this assay. Thirty-three samples measured less than the lowest standard, 3.13 pg/mL. Seven samples measured between 3.13 and 12.5 pg/mL.

Cell culture supernates - Human peripheral blood mononuclear cells ($1x10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate and stimulated for 1, 3, and 5 days with 10 μg/mL PHA. Aliquots of the culture supernate were removed on days 1, 3, and 5 and assayed for levels of natural IL-6. Results are listed in the following table.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	575	311	660
Stimulated	17,130	17,520	16,340

SPECIFICITY

This assay recognizes both natural and recombinant human IL-6. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5A and at 100 ng/mL in Calibrator Diluent RD6F, and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-6 control prepared in Calibrator Diluent RD5A and 100 ng/mL in a mid-range rhIL-6 control prepared in Calibrator Diluent RD6F were assayed for interference. No significant cross-reactivity or interference was observed.

Factors related to or associated with IL-6:

Recombinant human:
IL-6 sR
IL-6 sR/sgp 130
IL-11
IL-12
CNTF
G-CSF
sgp130
LIF
LIF R
OSM
Recombinant mouse:
IL-6
IL-11
IL-12
Other:
rrCNTF

Other factors:

Recombinant human:
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-7
IL-8
GM-CSF
LIF
TNF-α
TNF-β
Recombinant mouse:
IL-2
IL-3
IL-4
IL-5
IL-7
GM-CSF
Other:
bFGF acidic
bFGF basic
hPDGF
pPDGF
hTGF-β1
pTGF-β1.2
pTGF-β2

REFERENCES

1. Kishimoto, T. *et al.* (1992) Science **258**:5593.
2. Kishimoto, T. (1992) Int. Arch. Allergy Immunol. **99**:172.
3. Hirano, T. *et al.* (1990) Immunol. Today **11**:443.
4. Hirano, T. (1992) Clin. Immunol. and Immunopathol. **62**:S60.
5. Hirano, T. *et al.* (1990) in *Peptide Growth Factors and their Receptors I*, Sporn, M.B. and A.B. Roberts eds., Springer-Verlag, New York, p. 663.
6. Hirano, T. *et al.* (1986) Nature **324**:73.
7. Haegeman, G. *et al.* (1986) Eur. J. Biochem. **159**:625.
8. Zilberstein, A. *et al.* (1986) EMBO J. **5**:2529.
9. May, L.T. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:8957.
10. Van Snick, J. *et al.* (1988) Eur. J. Immunol. **18**:193.
11. Yasukawa, Y. *et al.* (1987) EMBO J. **6**:2939.
12. Okada, M. *et al.* (1983) J. Exp. Med. **157**:583.
13. Butler, L. *et al.* (1984) Proc. Natl. Acad. Sci. USA **81**:2475.
14. Hirano, T. *et al.* (1984) J. Immunol. **133**:798.
15. Kikutani, H. *et al.* (1985) J. Immunol. **134**:990.
16. Lotz, M. *et al.* (1988) J. Exp. Med. **167**:1253.
17. Tosato, G. and S.E. Pike (1988) J. Immunol. **141**:1556.
18. Uttenhove, C. *et al.* (1988) J. Exp. Med. **167**:1417.
19. Bauman, H. *et al.* (1984) J. Biol. Chem. **259**:7331.
20. Wong, G.G. *et al.* (1988) J. Immunol. **140**:3040.
21. Leary, A.G. *et al.* (1988) Blood **71**:1759.
22. Van Damme, J. *et al.* (1988) J. Immunol. **140**:1534.
23. Nordan, R.P. *et al.* (1987) J. Immunol. **139**:813.
24. Tosato, G. *et al.* (1988) Science **239**:502.
25. Shabo, Y. *et al.* (1988) Blood **72**:2070.
26. Satoh, T. *et al.* (1988) Mol. Cell. Biol. **8**:3546.
27. Hama, T. *et al.* (1989) Neurosci. Letts. **104**:340.
28. Ishabashi, T. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:5953.
29. Madhok, R. *et al.* (1993) Ann. Rheum. Dis. **52**:232.
30. Wendling, D. *et al.* (1993) J. Rheumatol. **20**:259.
31. Grossman, R.M. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:6367.
32. Hyams, J.S. *et al.* (1993) Gastroenterol. **104**:1285.
33. Dunbar, C.E. and A.W. Nienhuis (1993) JAMA **269**:2412.
34. Kurzrock, R. *et al.* (1993) Cancer Res. **53**:2118.
35. Watson, J.M. *et al.* (1993) Gynecol. Oncol. **49**:8.
36. Bendtzen, K. *et al.* (1990) Immunol. Today **11**:167.
37. Hansen, M.B. *et al.* (1992) Cytokine **5**:72.
38. Rose, T.M. and A.G. Bruce (1991) Proc. Natl. Acad. Sci. USA **88**:8641d.
39. Bazan, J.F. (1991) Neuron **7**:197.
40. Gaines Das, R.E. and S. Poole (1993) J. Immunol. Meth. **160**:147.

NOTES