Mouse GM-CSF ELISA Kit

For the quantitative determination of mouse granulocyte macrophage colony stimulating factor (GM-CSF) concentrations in serum, cell culture supernatant, and other biological fluids

Catalogue Number: MEC1004

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

S7.5 (01) mGM-CSF

TABLE OF CONTENTS

INTENDED USE

This Mouse GM-CSF ELISA Kit is to be used for the *in vitro* quantitative determination of mouse granulocyte macrophage colony stimulating factor (GM-CSF) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH ONLY.

INRODUCTION

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is a member of the hematopoietic cytokine family, which includes interleukin-3 (IL-3) and interleukin-5 (IL-5). The non-glycosylated recombinant mouse GM-CSF has a calculated molecular weight of 14kDa. Because of the formation of intramolelular disulfide bonds and glycosylation, GM-CSF migrates at different sizes on SDS PAGE gel. Human GM-CSF shows 56-60% amino acid homology to murine GM-CSF but does not exhibit cross-species biological activity or receptor binding.

GM-CSF is a potent stimulator to the marrow granulocyte/monocyte progenitor cells and promotes the function of mature granulocytes and monocytes. GM-CSF is synthesized upon stimulation by inflammatory agent or antigen. GM-CSF can be produced by and act upon a variety cell types, including T-lymphocytes, monocytes/macrophages, B-lymphocytes, endothelial cells, fibroblasts, stromal cells, mesothelial cells, keratinocytes, osteoblasts, uterine epithelial cells, synoviocytes, mast cells and various solid tumors.

Mouse GM-CSF receptor is comprised of a unique alpha subunit and a common beta subunit which is shared by IL-3 and IL-5 receptor. GM-CSF receptor is abundant on progenitor and mature granulocytes and monocytes. The low level expression of GM-CSF receptor on non-hematopoietic cells may explain the phenomenon that higher GM-CSF concentration is required for producing biological effects on these cells.

GM-CSF stimulates macrophage production of TNF, M-CSF, G-CSF, and IL-1, and promotes the killing of infectious agents by granulocytes and macrophages.

Increased GM-CSF levels were found to be associated with various inflammatory and pathological conditions including pneumonia, asthma, psoriasis, pulmonary fibrosis, rheumatoid arthritis, systemic lupus erythematosus, myelodysplastic syndrome (MDS), thrombocytopenia, lung cancer, acute mylogenous leukemia, and tumour related thrombocytosis,

GM-CSF showed therapeutic value in accelerating neutrophil recovery at myelo-suppression conditions, such as bone marrow transplantation, chemotherapy, and infectious disease.

This ELISA kit provides a tool for studying GM-CSF expression and regulation in animal model.

PRINCIPLE OF THE ASSAY

This mouse GM-CSF enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for mouse GM-CSF. Standards or samples are then added to the appropriate microtiter plate wells and incubated. Mouse GM-CSF, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound mouse GM-CSF and other components of the sample. In order to quantitatively determine the amount of mouse GM-CSF present in the sample, a standardized preparation of horseradish peroxidase HRP-conjugated monoclonal antibody specific for mouse GM-CSF is added to each well to "sandwich" the mouse GM-CSF immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain mouse GM-CSF and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 $\pm$ 2 nm.

In order to measure the concentration of mouse GM-CSF in the samples, this kit includes two diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing). According to the testing system, the provided standard is diluted (2-fold dilution series) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus mouse GM-CSF concentration (pg/mL). The concentration of mouse GM-CSF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This mouse GM-CSF ELISA is a 3.5-hour solid phase immunoassay readily applicable to measure mouse GM-CSF in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 3200 pg/mL. It showed no cross reactivity with other cytokines tested.

REAGENTS PROVIDED
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **Mouse GM-CSF MICROTITER PLATE** (Part MEC04-1) ***96 wells***
   Pre-coated with anti-mouse GM-CSF monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part MEC04-2) ***6 mL***
   Anti-mouse GM-CSF monoclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part MEC04-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **GM-CSF STANDARD** (Part MEC04-4) ***2 vials***
   Recombinant mouse GM-CSF (3.2 ng/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC04-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part MEC04-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing*.

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part MEC04-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 µL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm±2 nm)
8. Automatic microtiter plate washer or squirt bottle
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate mouse viruses.
   Solid Wastes: Autoclave 60 min. at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone; keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING, AND STORAGE

a). **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.

b). **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, cell culture, and cell culture supernatant samples to be used within 24-48 hour may be stored at 2-8°C。 Otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable 1 month at 2-8°C . Mix well before use.

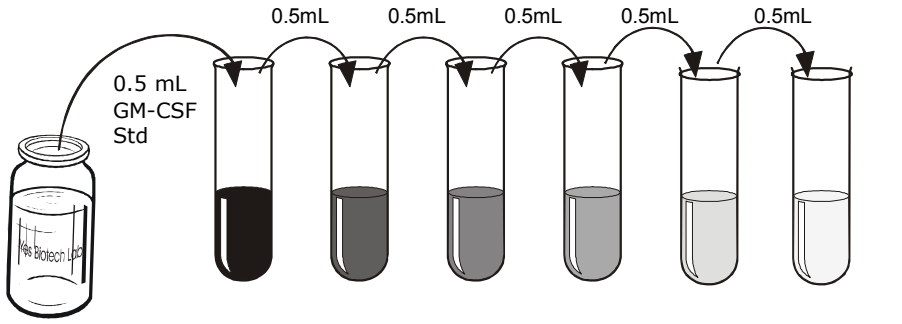
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	3.0	3.0	6.0
4 strips (32 wells)	6.0	6.0	12.0
6 strips (48 wells)	8.0	8.0	16.0
8 strips (64 wells)	10.0	10.0	20.0
10 strips (80 wells)	12.0	12.0	24.0
12 strips (96 wells)	14.0	14.0	28.0

3. **<u>Mouse GM-CSF Standard</u>:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the mouse GM-CSF Standard with either 1.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 3200 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The mouse GM-CSF standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (50 to 3200pg/mL) as illustrated below. Add 0.5 mL of the <u>appropriate</u> <u>Calibrator</u> <u>Diluent</u> to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The diluted GM-CSF Standard will serve as the high standard (3200 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer and mouse GM-CSF Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided by used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0 pg/mL**	(S1)	**2C, 2D**	Standard 6 - **800 pg/mL**	(S6)
1C, 1D	Standard 2 - **50 pg/mL**	(S2)	**2E, 2F**	Standard 7 -**1600pg/mL**	(S7)
1E, 1F	Standard 3 – **100 pg/mL**	(S3)	**2G, 2H**	Standard 8 -**3200pg/mL**	(S8)
1G, 1H	Standard 4 – **200 pg/mL**	(S4)	**3A-12H**	GM-CSF samples	
2A, 2B	Standard 5 – **400 pg/mL**	(S5)			

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50µL of Standard or Sample to the appropriate well of the antibody pre-coated wells of the Microtiter Plate. Cover and incubate for 1 hours at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

4. Add 50µL Anti-mouse GM-CSF Biotin conjugate to each well. Cover and incubate for 1 hour at room temperature.

5. Repeat wash procedure as described in Step 3.

6. Add 100µL avidin Conjugate to each well. Cover and incubate for 1 hour at room temperature.

7. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

8. Repeat wash procedure as described in Step 3.

9. Add 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

10. Add 100µL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of mouse GM-CSF in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding GM-CSF concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of mouse GM-CSF in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding GM-CSF concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

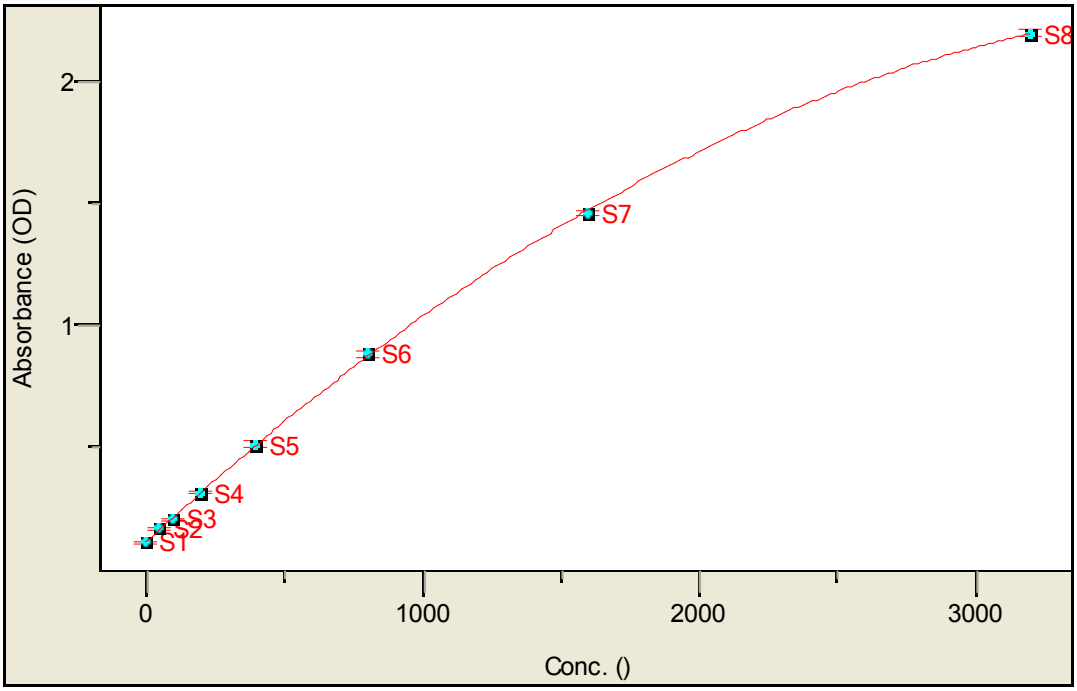
TYPICAL DATA

Results of a typical standard run of a mouse GM-CSF ELISA are shown. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain own standard curve.

EXAMPLE

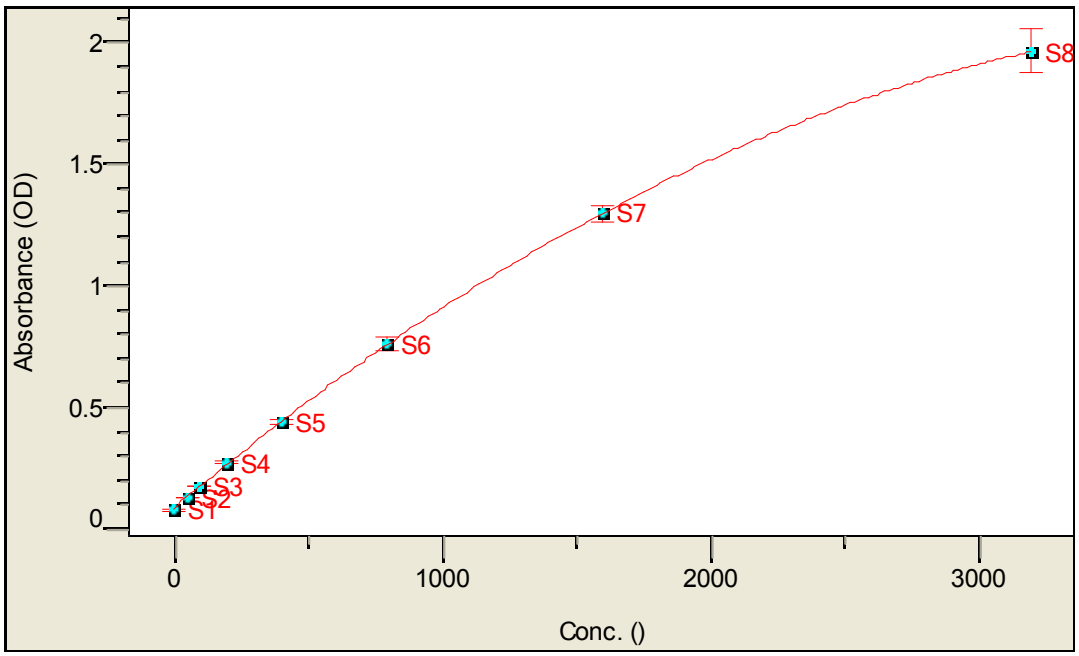
1. The following data was obtained for a standard curve using Calibrator Diluent I.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.) - (S1)
0	0.095	3.23	0
50	0.1555	4.70	0.06
100	0.1970	2.05	0.102
200	0.3055	1.57	0.2105
400	0.4990	2.78	0.404
800	0.8725	1.68	0.7775
1600	1.4520	6.77	1.357
3200	2.1855	6.77	2.0905



2. The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.) - (S1)
0	0.085	2.6	0
50	0.134	0	0.049
100	0.1805	1.18	0.0956
200	0.2775	1.27	0.1925
400	0.4435	3.03	0.3585
800	0.7645	3.61	0.6795
1600	1.300	2.50	1.215
3200	1.9675	4.49	1.8825



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by replicates of sixteen in 1 assay.

Sample	**1**	**2**	**3**
N	16	16	16
Mean (pg/ml)	109	1208	2673
Coefficient of Variation (%)	4.22	3.10	3.56

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of know concentrations were assayed by replicates on 10 different assays.

Sample	**1**	**2**	**3**
N	10	10	10
Mean (pg/mL)	114	1166	2328
Coefficient of Variation (%)	6.30	3.21	6.98

3. **RECOVERY** The recovery of GM-CSF spiked to different levels throughout the range of the assay was evaluated. All samples were mixed and assayed in duplicate.

Sample Type	Average Recovery %	Range %
Cell culture media	102.5	93-114
Mouse Serum	82%	81-83%

4. **SENSITIVITY**
The minimum detectable dose of GM-CSF was determined by adding two standard deviations to the mean optical density value of 24 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse GM-CSF calculated from calibrate II diluted standard curve was <17 pg/mL.

5. **SPECIFICITY**
This sandwich ELISA recognizes both natural and recombinant mouse GM-CSF. This kit exhibits no significant cross-reactivity with any other mouse cytokine.

REFERENCES

1. Wong et al. (1985) *Science.* 228: 810.
2. Thompson, A.W. (1994) *Cytokine Handbook* (eds J. Rasko). London: Academic Press, pp.343.
3. Hamblin, A.S. (1993) *Cytokines and Cytokine Receptors.* London: Oxford University Press, pp. 35.
4. DiPersio et al, (1988) *J. Biol. Chem*. 263 :1834
5. Robinson, B.E. et al (1987) *J. Clinical Inves.* 79: 1648.
6. Reid, C et al. (1993) *Dendritic Cells in Fundamental and Clinical Immunology*, (eds Kamperdijk). New York: Plenum Press, pp. 257.
7 Lafeuillade, A. et al. (1996) *Lancet.* 347: 1123.
8. Thacker, D. et al. (1994) *Int. J. Cancer.* 56: 236.
9. Xing, Z. et al. (1996) *J. Leukocy Biol.* 59: 481.
10. Xing, Z. (1996) *J. Clinical Invest..* 97: 1102.
11. Joost van Pelt, L et al. (1996) *Blood.* 87(12): 5306.
12. Sawyer, C.L et al (1992) *Cancer.* 69: 1342.
13. Omori, F et al. (1992) *Biotherapy.* 4: 147.
14. Suzuki, A et al., (1992) *Blood*. 80: 2052.
15. Zwierina, H. et al. (1992). *Leuk. Res.* 16: 1181.
16. Abboud, M.R. (1996) *Br J. Haematol*. 92(2): 486.
17. Takematsu, H. and H. Tagami (1990) *Dermatologica.* 181: 16.
18. Nakamura, Y et al. (1993) *Am. Rev Respir. Dis*. 147: 87.
19. Argo, A, (1992). *J. Rheumatol*. 19. 1065.
20. Prince, H.M. et al (1995). *Bone Marrow Transplant*. 6(1): 195.
21. Shaarawy, M. (1995) *Cytokine.* 7(2): 171.