Human MCP-3 ELISA Kit

For the Quantitative Determination of Human Monocyte Chemotactic Protein-3 (MCP-3) Concentrations in Serum, Plasma, Cell Culture Supernatant, and Other Biological Fluids.

Catalogue Number: EL10016

96 tests

FOR LABORARTORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTENDED USE

This Human MCP-3 ELISA Kit is to be used for the *in vitro* quantitative determination of human monocyte chemotactic protein-3 (MCP-3) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Monocyte Chemotactic Protein (MCP-3) is a novel chemokine that has been recently purified from human osteosarcoma cell line[1]. It was shown that MCP-3 is produced by both tumours cells[1] and leukocytes.[2,3] MCP-3 can bind and activate a vast diversity of inflammatory cell types through interaction with multiple leukocyte receptors as well as it's own receptor.[6,7] Murine MCP-3 Marc/Fic chemokines that have been previously cloned are thought to be homologues of human MCP-3.[3,4] MCP-3 cDNA cloning[2] and structural analysis revealed that this 76 amino acid (a.a.) polypeptide with a molar mass of 8.5 kDa belongs to a family of small inflammatory proteins, characterized by four conserved cysteine residues and is localized on human chromosome 17.[1,4,5] MCP-3 is designated a C-C or intercrine ß cytokine.[13] MCP-3 can indeed use a wide variety of binding sites and activate many inflammatory cells. *In vitro*, both MCP-3 and MCP-1 can activate T-cells, monocytes, and basophils, but only the first can activate eosinophils. These cytokines show 71% a.a. homology. MCP-3 was also revealed to have 30% a.a homology with MIP-1 and RANTES which can also activate eosinophils plus all cell types mentioned above.[6,7] MCP-3 seems to be the only C-C chemokine that regularly induces neutrophil migration. It is also a potent chemoattractant for human dendritic cells.[7]

It has been suggested that MCP-3 binds multiple C-C receptors such as MCP-1 on monocytes and basophils [8,9], MIP-1a on neutrophils, basophils, and eosinophils[9] and RANTES on basophils and eosinophils[(9,10)]. Evidence suggests that MCP-3 does indeed use multiple receptors (MCP-1, RANTES, and MIP-1a) and binds with MIP-1ß receptor as well as other unique binding sites [6,7]. MCP-3 was discovered to be the strongest C-C chemokine in inducing the migration of C-C CKR1 transfected cells. It was shown that MCP-3 binds with C-C CKR1 receptor with greater affinity than MIP-1a or Rantes, which mainly activate it [6,11]. Also, MCP-3 promotes exocytosis of eosinophil granule proteins and stimulates histamine release from human basophils [4,12]. *In vivo*, MCP-3 induces the selective infiltration of monocytes on intradermal injection in rabbits.[1] Since MCP-3 acts on a variety of inflammatory cells and utilizes multiple receptors for its function, characterization and isolation of the shared as well as unique receptors for MCP-3 will provide further insights into the pathophysiological roles of MCP-3. C-C chemokines are mediators of a number of pathological conditions such as chronic inflammation, tumor, allergy, as well as atherosclerosis[10]. Since the binding and signaling of MCP-3 is most promiscuous, the development of antibody or antagonist which can block MCP-3 through binding to the receptor or ligand may prove to be useful in the treatment of diseases mediated by a number of C-C chemokine.

This MCP-3 ELISA is a 2.5 hour solid phase immunoassay readily applicable to measure MCP-3 in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1600 pg/mL. It showed no cross-reactivity with other cytokines tested. MCP-3 may play a role in certain diseases, therefore this MCP-3 ELISA is expected to be effectively used for further investigations into the relationship between MCP-3 and various pathological conditions.

PRINCIPLE OF THE ASSAY

This MCP-3 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 MCP-3. Standards or samples are then added to the appropriate microtiter plate wells and incubated. MCP-3, if present, will bind and become immobilised by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound MCP-3 and other components of sample. In order to quantitate the amount of MCP-3 present in the sample, a standardised preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific for MCP-3 is added to each well to "sandwich" the MCP-3 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 MCP-3 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 nm ± 2 nm.

In order to measure the concentration of MCP-3 in the samples, this kit includes two calibration 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) with 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 MCP-3 concentration (pg/mL). The concentration of MCP-3 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

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

	96 tests

1. **MCP-3 MICROTITER PLATE** (Part EL16-1) ***96 wells***
   Pre-coated with anti-human MCP-3 monoclonal antibody.

2. **MCP-3 CONJUGATE** (Part EL16-2) ***12 mL***
   Anti-human MCP-3 monoclonal antibody conjugated to horseradish peroxidase with preservative.

3. **MCP-3 STANDARD** (Part EL16-3) ***2 vials***
   Recombinant human MCP-3 (6.4 ng/vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT I** (Part EL16-4) ***25 mL***
   Animal serum with preservative. *For serum/plasma testing.*

5. **CALIBRATOR DILUENT II** (Part EL16-5) ***25 mL***
   Cell culture medium with calf serum and preservative.

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

7. **SUBSTRATE A** (Part EL16-7) ***10 mL***
   Buffered solution with H_2O_2

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

9. **STOP SOUTION** (Part 30008) ***14 mL***
   2N Suphuric 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. Human 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 human 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 human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: 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 viruses 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

1. 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.

c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Samples should be used immediately. Otherwise, samples must be aliquoted and 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.

2. DILUTION PROCEDURES

Serum/plasma samples: Do not require any dilution to detect normal MCP-3 concentrations.

Cell culture supernatant samples: Require a final, in well dilution of 1:2 (equal volume) in order to detect normal MCP concentrations. *Dilute samples with Sample Diluent* (see Assay Procedure).

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 for 1 month at 2-8°C. Mix well before use.

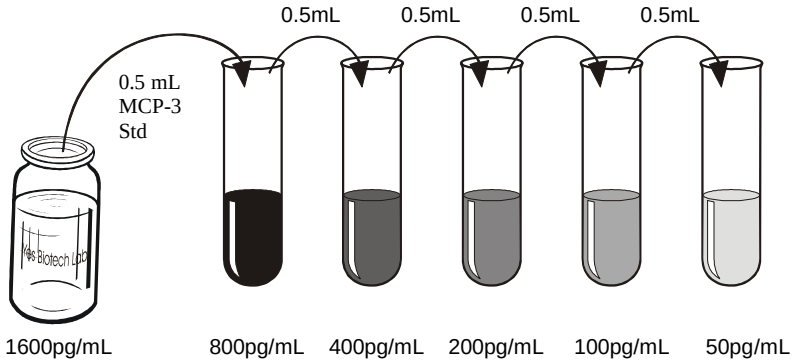
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **MCP-3 Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute MCP-3 Standard with either 4.0mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 1600 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 MCP-3 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 1600 pg/mL) as illustrated. Add 0.5mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted MCP-3 Standard will serve as the high standard (1600 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer and MCP-3 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be 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)	**2A, 2B**	Standard 5 - **400 pg/mL** (S5)
1C, 1D	Standard 2 - **50 pg/mL** (S2)	**2C, 2D**	Standard 6 - **800 pg/mL** (S6)
1E, 1F	Standard 3 - **100 pg/mL** (S3)	**2E, 2F**	Standard 7 - **1600 pg/mL** (S7)
1G, 1H	Standard 4 - **200 pg/mL** (S4)	**2G-12H**	MCP-3 samples

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100µL of Standards and samples to the appropriate well of the appropriate the antibody pre-coated Microtiter Plate, than, cover and incubate for **1 hour 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 350µL/well/wash (range: 350-400µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Dispense 100μL of conjugate into each well. Cover and incubate for **1 hour at room temperature.**

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

6. Repeat wash procedure as described in Step 3.

7. Dispense 100 μL Substrate Solution to each well. Cover and incubate for **15 minutes at room temperature.**

8. Add 100 μL 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

The standard curve is used to determine the amount of MCP-3 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 MCP-3 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 value of the zero-standard (0 pg/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of MCP-3 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 MCP-3 concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay. the concentration read from the standard curve must be multiplied by the dilution factor.

3. To determine the final concentration of MCP-3 in cell culture medium sample, the concentration read from the standard curve must be multiplied by the *dilution factor (2)*

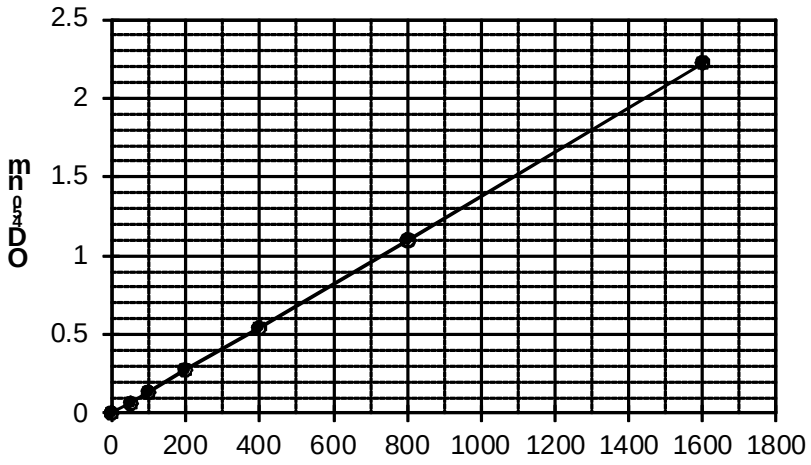
TYPICAL DATA

Results of a typical standard run of a MCP-3 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 their own standard curve.

EXAMPLE ONE

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

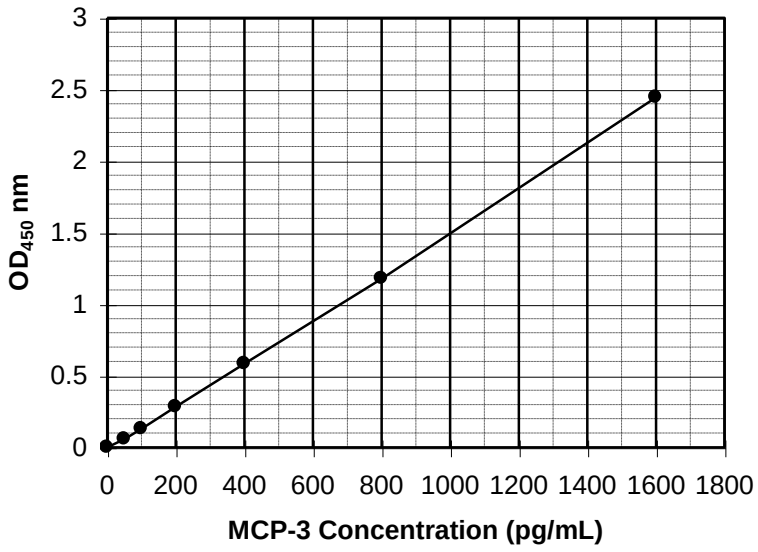
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.028, 0.032	0.030	0
50	0.100, 0.098	0.099	0.069
100	0.170, 0.168	0.169	0.139
200	0.310, 0.308	0.309	0.279
400	0.571, 0.570	0.571	0.541
800	1.142, 1.122	1.132	1.102
1600	2.260, 2.250	2.255	2.225



EXAMPLE TWO

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

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.030, 0.032	0.031	0
50	0.085, 0.090	0.088	0.057
100	0.160, 0.162	0.161	0.130
200	0.310, 0.320	0.315	0.284
400	0.620, 0.610	0.615	0.584
800	1.207, 1.210	1.209	1.178
1600	2.472, 2.480	2.476	2.445



PERFORMANCE CHARACTERISTICS

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

	Calibrator Diluent I Assay		*Calibrator Diluent II Assay*		
Sample	**1**	**2**	**1**	**2**	**3**
n	20	20	10	13	20
Mean (pg/mL)	213.7	958.3	64.8	232.7	1215.9
Standard Deviation (pg/mL)	10.6	30.3	6.4	15.4	38.2
Coefficient of Variation (%)	*5.0*	*3.2*	*9.9*	*6.6*	*3.1*

2. **INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of known concentration were assayed by replicates on 20 different assays.

	Calibrator Diluent I Assay		***Calibrator Diluent II Assay***		
Sample	**1**	**2**	**1**	**2**	**3**
n	20	20	20	20	20
Mean (pg/mL)	204.5	986.3	61.8	208.5	1037.3
Standard Deviation (pg/mL)	13.5	44.6	6.3	13.1	58.4
Coefficient of Variation (%)	*6.6*	*4.5*	*10.2*	*6.3*	*5.6*

3. **RECOVERY**

The recovery of MCP-3 spiked to 3 different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture media	102.1	99.4 – 105.3
Serum	102.0	98.8 – 106.7
Plasma	101.4	99.6 – 104.6

4. **SENSITIVITY**

The minimum detectable dose of MCP-3 was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is *30 pg/mL* and using Calibrator Diluent II is *20 pg/mL.*

5. **SPECIFICITY**

This sandwich ELISA can recognises both natural and recombinant human MCP-3. This kit exhibits no significant cross-reactivity with human IL-8, IL-1ß, SAA, RANTES, MCAF, EGF, TNF-α, TGF-ß, M-CSF, GM-CSF, FGF, and EPO.

6. **CALIBRATION**

This immunoassay is calibrated against a highly purified E. Coli-expressed 76-amino acid polypeptide form of recombinant human MCP-3.

7. **EXPECTED NORMAL VALUES**

Fourteen apparently healthy, normal individuals were evaluated in this assay. The MCP-3 concentration of serum/plasma samples were less than 30 pg/mL. The MCP-3 concentration of urine samples were less than 20 pg/mL.

REFERENCES

1. Van Damme, J. et al. (1992) *J. Exp. Med.* 176: 59
2. Opdenakker, G.,et al.(1993) *Biochem. Biophys. Res. Commun.* 191: 535.
3. Minty, A. et al, 1993. *Eur. Cytokine Netw*. 4, 99-110.
4. Thirion, S. et al. (1994) *Biochem. Biophy. Res. Comm*. 201: 493.
5. Opdenakker, G. et al. (1994) *Genomics*. 21: 403.
6. Ben-Baruch, S. et al. (1995) *J. of Biol. Chem*. 270 (38): 22123.
7. Xu, L.L. et al. (1995) *Eur. J. Immun.* 25: 2612.
8. Sozzani, S. et al. (1994) *Immunol*. 152: 3615.
9. Dahinden, C.A. et al. (1994) *J. Exp. Med*. 179: 751.
10. Noso, N. et al. (1994) *Biochem. Biophys. Res. Comm*. 200: 1470.
11. Jiang-Hong, G. (1996). *J. Biological Chemistry.* 27*(*18):10521.
12. Ying, S. et al. (1995) *J. Exp. Med*. 181: 2153.
13. Baggiolini, M. et al. (1994) *Advances in Immunology.* 55:97.
14. Nasur, S. et al (1995). *J. of Interferon and Cytokine Res.* 15:955