

Quantikine™ ELISA

Human CCL11/Eotaxin Immunoassay

Catalog Number DTX00

STX00

PDX00

For the quantitative determination of human Eotaxin 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.

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INTRODUCTION

Human Eotaxin is an 8.3 kDa, 74 amino acid (aa), non-glycosylated polypeptide that is a member of the CC family of chemokines (1, 2). Human Eotaxin exhibits considerable species cross-reactivity (3, 4), showing approximately 60% aa sequence identity with both mouse and guinea pig Eotaxins (2, 5, 6). In comparison with other human CC chemokines, Eotaxin shows 66% aa sequence identity with MCP-1 (1) and 58% aa sequence identity with MCP-4 (7). Eotaxin shows only 39% aa sequence identity with Eotaxin-2, a molecule named for its biological activity rather than for structural or sequence similarity to Eotaxin (8). Although Eotaxin may be constitutively produced (9), it appears most often to be induced by inflammatory cytokines such as IL-1, TNF- α and IFN- γ (5, 9-11). Cells reported to produce eotaxin are quite varied in type, including endothelial cells (2, 5, 12), fibroblasts (9), macrophages (2, 12), ciliated and non-ciliated bronchial epithelial cells (2, 12), smooth muscle cells (2, 12), chondrocytes (12), and eosinophils (2, 10).

Eotaxin has been shown to be a potent and selective chemoattractant for eosinophils during inflammation and allergic reactions (13). The activities of Eotaxin have been shown to be mediated by the human receptor CCR3, the third of eight currently known CC family G-protein coupled chemokine receptors (14-18). CCR3 is a serpentine 45-55 kDa, 355 aa protein that is characterized by the presence of seven transmembrane domains (19, 20). Although CCR3 is the only known receptor for Eotaxin ($K_d = 100-500$ pM), other chemokines do bind to CCR3, including Eotaxin-2 and MCP-4 (with high affinity), MCP-3, leukotactin-1, and RANTES (with lower affinity) (7, 8, 19, 21). Notably, modest changes in local pH and ionic strength markedly impact eotaxin binding to CCR3, suggesting that small perturbations of the tissue micro-environment may represent an additional mechanism for regulating the biological effects of Eotaxin (22). Cells known to express CCR3 include eosinophils (20), basophils (23, 24) and Type 2 T helper cells (Th2) (25).

The Quantikine™ Human CCL11/Eotaxin Immunoassay is a 3.5 hour solid phase ELISA designed to measure human Eotaxin in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Eotaxin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human Eotaxin accurately. Results obtained measuring natural human eotaxin showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human Eotaxin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Eotaxin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Eotaxin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Eotaxin 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 Eotaxin 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.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DTX00	CATALOG # STX00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Eotaxin Microplate	890584	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Eotaxin.	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.*
Human Eotaxin Standard	890586	2 vials	12 vials	Recombinant human Eotaxin in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Discard the Eotaxin stock solution after 4 hours. Use a fresh standard for each assay.
Human Eotaxin Conjugate	890585	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human Eotaxin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. <i>For cell culture supernate samples.</i>	
Calibrator Diluent RD6O	895120	1 vial	6 vials	21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples. May contain a precipitate. Mix well before and during use.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DTX00 contains sufficient materials to run an ELISA on one 96 well plate.

STX00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDTX00). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
Note: Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human Eotaxin Microplate	890584	50 plates
Human Eotaxin Standard	890586	50 vials
Human Eotaxin Conjugate	890585	50 vials
Assay Diluent RD1W	895117	50 vials
Calibrator Diluent RD5K	895119	50 vials
or		
Calibrator Diluent RD60	895120	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package inserts	750304	2 booklets

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
- Test tubes for dilution of standards.
- Human Eotaxin Controls (optional; R&D Systems®, Catalog # QC21)

PRECAUTIONS

Calibrator Diluent RD6O 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 preservatives 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. Refer to the SDS 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 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 at room temperature before centrifugation for 15 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 or citrate as an anticoagulant. Centrifuge for 15 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 plasma are not recommended for use in this assay.
Hemolyzed samples are not suitable for use in this assay.*

REAGENT PREPARATION

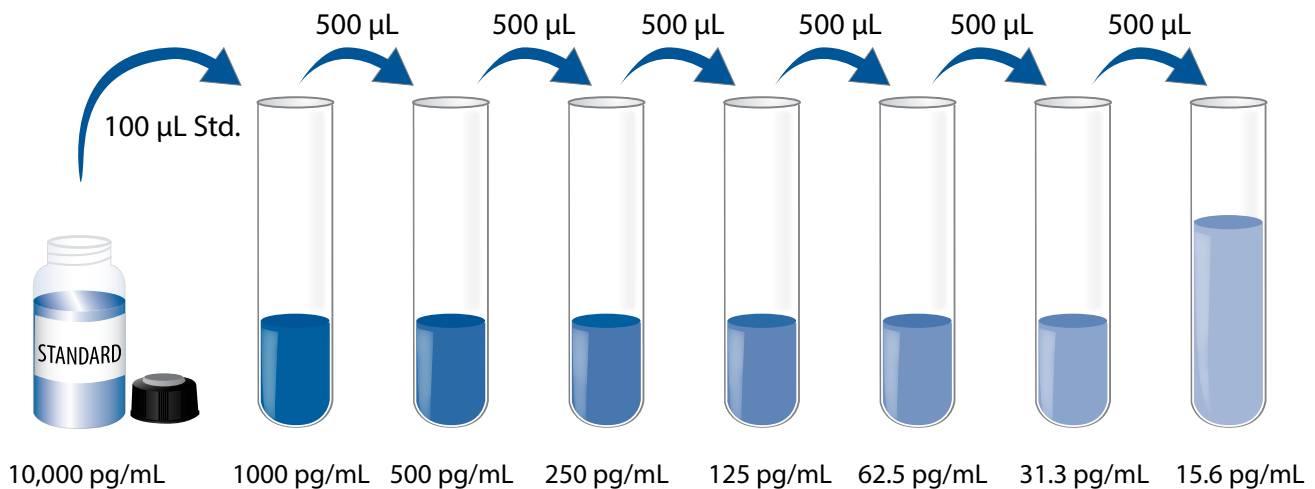
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of 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.

Human Eotaxin Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human Eotaxin Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μL of the Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6O (*for serum/plasma samples*) into the 1000 pg/mL tube. Pipette 500 μL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. 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 standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, 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 50 μL of standard, control, or sample per well. 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 twice for a total of three 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 Human Eotaxin Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the 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.

CALCULATION OF RESULTS

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

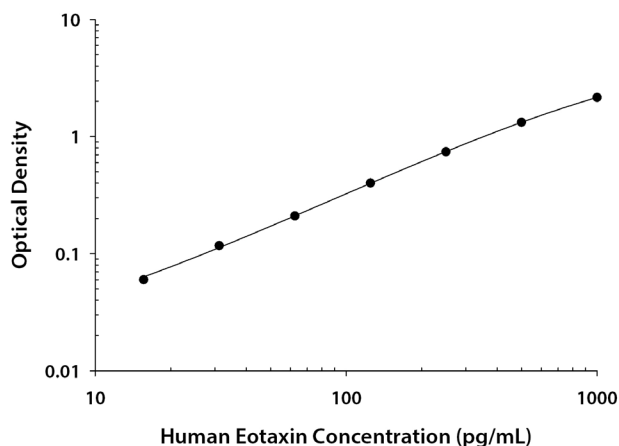
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

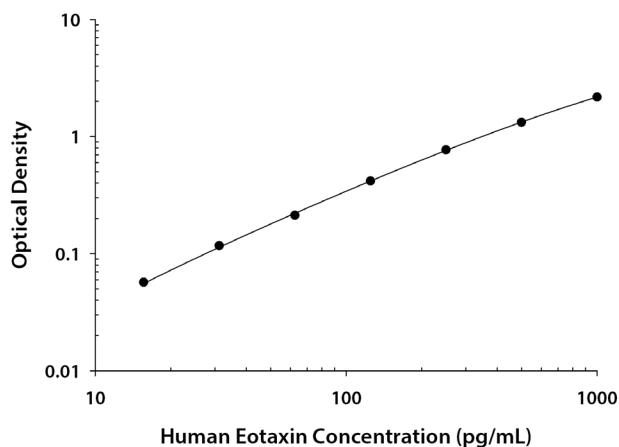
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.023	0.024	—
15.6	0.084 0.085	0.084	0.060
31.3	0.144 0.138	0.141	0.117
62.5	0.235 0.234	0.234	0.210
125	0.425 0.426	0.425	0.401
250	0.760 0.771	0.766	0.742
500	1.343 1.356	1.350	1.326
1000	2.219 2.144	2.182	2.158

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.029 0.029	0.029	—
15.6	0.084 0.089	0.086	0.057
31.3	0.148 0.144	0.146	0.117
62.5	0.239 0.246	0.242	0.213
125	0.444 0.447	0.446	0.417
250	0.778 0.817	0.798	0.769
500	1.347 1.362	1.354	1.325
1000	2.159 2.247	2.203	2.174

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	66.8	184	390	67.5	193	396
Standard deviation	2.0	8.4	23.5	7.8	18.2	40.2
CV (%)	3.0	4.6	6.0	11.6	9.4	10.2

SERUM/PLASMA ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	64.5	109	377	63.4	190	400
Standard deviation	2.5	5.8	12.8	7.3	19.6	33.5
CV (%)	3.9	5.3	3.4	11.5	10.3	8.4

RECOVERY

The recovery of human Eotaxin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	100	86-111%
Serum (n=5)	95	83-109%
EDTA plasma (n=5)	99	82-115%
Citrate plasma (n=5)	99	81-117%

SENSITIVITY

The minimum detectable dose (MDD) of human Eotaxin is typically less than 5 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess linearity of the assay, samples spiked with high concentrations of human Eotaxin were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	101	105	102	106
	Range (%)	89-113	98-109	90-112	102-113
1:4	Average % of Expected	99	103	101	106
	Range (%)	96-104	94-113	91-110	98-117
1:8	Average % of Expected	97	103	104	106
	Range (%)	91-101	93-114	93-117	99-116
1:16	Average % of Expected	98	101	97	105
	Range (%)	93-103	92-112	89-115	98-116

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Eotaxin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Eotaxin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=95)	163	50-642	78
EDTA plasma (n=34)	80	32-272	43
Citrate plasma (n=34)	132	47-399	81

Cell Culture Supernates - Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for human Eotaxin. All samples measured below the lowest Human Eotaxin Standard, 15.6 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Eotaxin.

Each of the factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Eotaxin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CCL1/I-309
CCL2/MCP-1
CCL3/MIP-1 α
CCL4/MIP-1 β
CCL5/RANTES
CCL7/MCP-3
CCL8/MCP-2
CXCL1/GRO α
CXCL2/GRO β
CXCL3/GRO γ
CXCL10/IP-10

Recombinant mouse:

CCL3/MIP-1 α
CCL4/MIP-1 β
CXCL1/KC

Recombinant mouse Eotaxin does not interfere but does cross-react approximately 0.03% in this assay.

REFERENCES

1. Kitaura, M. *et al.* (1996) *J. Biol. Chem.* **271**:7725.
2. Ponath, P.D. *et al.* (1996) *J. Clin. Invest.* **97**:604.
3. Griffiths-Johnson, D.A. *et al.* (1997) *Methods Enzymol.* **288**:241.
4. Teixeira, M.M. *et al.* (1997) *J. Clin. Invest.* **100**:1657.
5. Rothenberg, M.E. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* **92**:8960.
6. Jose, P.J. *et al.* (1994) *Biochem. Biophys. Res. Commun.* **205**:788.
7. Stellato, C. *et al.* (1997) *J. Clin. Invest.* **99**:926.
8. Forssmann, U. *et al.* (1997) *J. Exp. Med.* **185**:2171.
9. Bartels, J. *et al.* (1996) *Biochem. Biophys. Res. Commun.* **225**:1045.
10. Garcia-Zepeda, E.A. *et al.* (1996) *Nature Med.* **2**:449.
11. Lilly, C.M. *et al.* (1997) *J. Clin. Invest.* **99**:1767.
12. Li, D. *et al.* (1997) *Eur. Respir. J.* **10**:1946.
13. Gonzalo, J.A. *et al.* (1996) *J. Clin. Invest.* **98**:2332.
14. Murphy, P.M. (1996) *Cytokine Growth Factor Rev.* **7**:47.
15. Baba, M. *et al.* (1997) *J. Biol. Chem.* **272**:14893.
16. Yoshida, R. *et al.* (1997) *J. Biol. Chem.* **272**:13803.
17. Roos, R.S. *et al.* (1997) *J. Biol. Chem.* **272**:17251.
18. Tiffany, H.L. *et al.* (1997) *J. Exp. Med.* **186**:165.
19. Daugherty, B.L. *et al.* (1996) *J. Exp. Med.* **183**:2349.
20. Ponath, P.D. *et al.* (1996) *J. Exp. Med.* **183**:2437.
21. Youn, B-S. *et al.* (1997) *J. Immunol.* **159**:5201.
22. Dairaghi, D.J. *et al.* (1997) *J. Biol. Chem.* **272**:28206.
23. Ugucioni, M. *et al.* (1997) *J. Clin. Invest.* **100**:1137.
24. Yamada, H. *et al.* (1997) *Biochem. Biophys. Res. Commun.* **231**:365.
25. Sallusto, F. *et al.* (1997) *Science* **277**:2005.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

NOTES

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