Quantikine®

Human IL-5 Immunoassay

Catalog Number D5000
S5000
PD5000

For the quantitative determination of human interleukin 5 (IL-5) concentrations in cell supernates, serum, plasma, and urine.

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

Interleukin 5 (IL-5) is a cytokine that exerts pleiotropic effects on B cells and cells of the eosinophilic lineage. It is produced mainly in T cells in response to stimulation by parasite-derived antigens and allergens. In addition, various transformed B-cells, Reed-Sternberg cells in Hodgkin's disease and activated eosinophils can express IL-5 mRNA. In the mouse system, IL-5 is also induced in mast cells after stimulation with allergen/IgE complex or calcium ionophores. The biology of IL-5 has been the subject of several reviews (1 - 4).

Native mouse IL-5, purified from the conditioned medium of T cell lines, is a glycosylated, disulfide-linked homodimer with an apparent molecular weight of 45 kDa. The genes for IL-5 have been mapped to human chromosome 5 and mouse chromosome 11, closely linked to the genes for IL-3, IL-4 and GM-CSF (1, 2). The cDNAs for human and mouse IL-5 encode precursor proteins with signal peptides that are cleaved to form mature IL-5 containing 115 and 113 amino acid residues, respectively. Human IL-5 is 70% identical at the amino acid level to mouse IL-5. Whereas mouse IL-5 and human IL-5 are equally active on human cell lines, human IL-5 is much less active than mouse IL-5 in mouse cell assays (3).

IL-5 exerts its activity on target cells by binding to specific cell surface receptors. The components of the high-affinity receptor for both human and mouse IL-5 have been cloned (4). The IL-5 high affinity receptor complex is a heterodimer consisting of a low affinity α-subunit and a β-subunit that binds IL-5 only upon association with the α-subunit. The β-subunit of the IL-5 high affinity receptor complex is identical to the β-subunit of the IL-3 and GM-CSF high affinity receptor complexes. Both the α- and β-subunits are members of the cytokine receptor superfamily. In addition to the membrane-anchored IL-5 receptor α-subunit, cDNAs encoding two soluble isoforms of IL-5 Rα have been isolated from mouse and human cells. In human eosinophils and eosinophilic HL-60 cells, mRNA for the soluble IL-5 Rα is highly abundant. Soluble IL-5 Rα functions as an IL-5 antagonist *in vitro* (5).

Despite the fact that mouse IL-5 was initially discovered as a B-cell growth and differentiation factor, the exact role for IL-5 on human B cells has been controversial (1 - 4). Studies with purified human B-cells suggest that human IL-5 can increase Ig generation by B-cells in the presence of IL-2 (6) and augment IgM secretion by human B-cells activated with staphylococcal A Cowan I strain (7). In the human system, IL-5 functions primarily as a growth and differentiation factor on cells of the eosinophilic lineage. *In vitro*, IL-5 is chemotactic for eosinophils and can increase the survival of mature eosinophils as well as stimulate eosinophil functions. IL-5 is also a late-acting eosinophil differentiation factor that synergizes with various colony-stimulating factors to increase eosinophil progenitor production and eosinophil expansion. IL-5 has also been reported to be a basophil differentiation factor that can modify basophil mediator production and release (1 - 4).

Based on the multiple functions of IL-5 on eosinophils *in vitro*, possible roles for IL-5 in physiological and pathological conditions associated with eosinophilia have been studied (2, 8 - 12). Elevated levels of serum IL-5 have been reported for some asthmatic patients (13, 14), for patients infused with IL-2 for cancer immunotherapy (15), and for a patient with episodic angioedema and eosinophilia syndrome (16).

Assay methods for IL-5, based on the mitogenic effects of IL-5 on responsive cell lines, *e.g.*, mouse BCL1 or human TF-1, are tedious to perform and not completely specific for IL-5. The Quantikine IL-5 Immunoassay is a 4.5 - 5.5 hour solid phase ELISA designed to measure IL-5 levels in cell culture supernates, serum, plasma and urine. It contains *Sf* 21-expressed recombinant human IL-5 and antibodies raised against recombinant human IL-5, and has been shown to quantitate accurately the recombinant factor. Results obtained using natural human IL-5 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine IL-5 kit can be used to determine relative mass values for natural IL-5.

The existence of naturally occurring soluble receptors for IL-5 suggest the potential for interference by these receptors with immunoassays for IL-5. It has been observed in our laboratories that the measurement of IL-5 is insensitive to the addition of the recombinant form of the IL-5 soluble receptors (IL-5 sRα, IL-5 sRβ). Therefore it is probable that experimental sample measurements reflect the total amount of IL-5 present, *i.e.*, the total amount of free IL-5 plus the amount of IL-5 initially bound to soluble receptors, if any are present in the samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-5 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-5 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-5 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-5 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 sources or lots.
- 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.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # D5000	Cat. # S5000
IL-5 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-5.	890199	1 plate	6 plates
IL-5 Conjugate - 21 mL/vial of polyclonal antibody against IL-5 conjugated to horseradish peroxidase, with preservatives.	890200	1 vial	6 vials
IL-5 Standard - 2.5 ng/vial of recombinant human IL-5 in a buffered protein base with preservatives, lyophilized.	890201	1 vial	6 vials
Assay Diluent RD1U - 6 mL/vial of a buffered protein base with preservatives. May appear cloudy and contain a precipitate. Mix well before and during use.	895138	1 vial	6 vials
Calibrator Diluent RD5-5 - 21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/urine samples.*	895485	1 vial	6 vials
Calibrator Diluent RD6-35 - 21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	895360	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	——	4 strips	24 strips

D5000 contains sufficient materials to run an ELISA on one 96 well plate.
S5000 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PD5000). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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="9">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD5-5</td></tr>
<tr><td>Calibrator Diluent RD6-35</td></tr>
<tr><td>Assay Diluent RD1U</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></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.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Human IL-5 Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6-35 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. Wear eye, hand, face, and clothing protection when using this material.

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 15 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 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.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

REAGENT PREPARATION

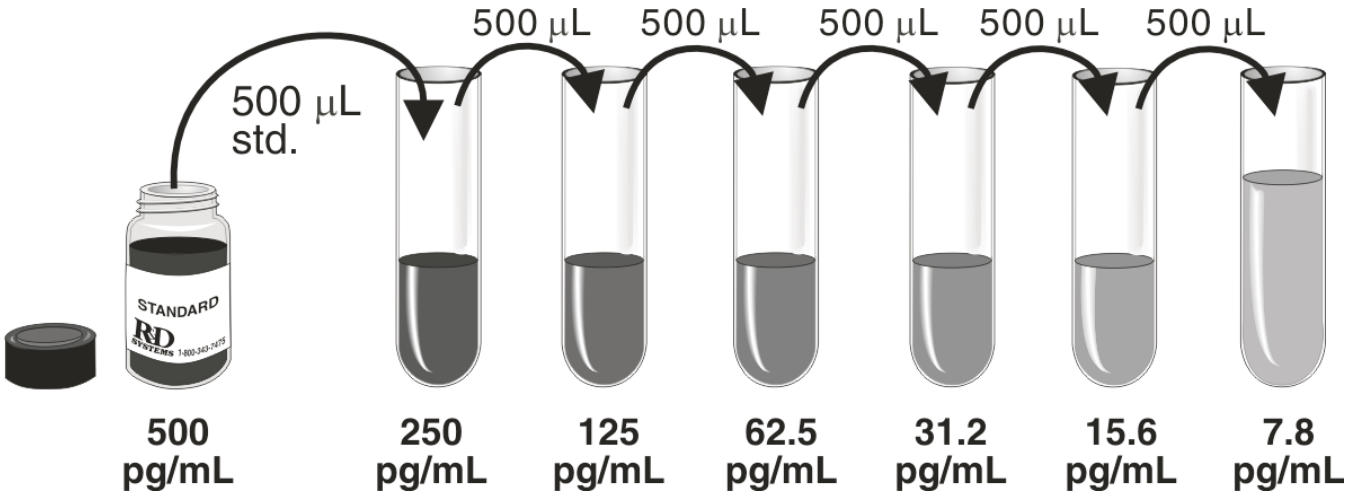
Bring all reagents to room temperature before use.

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-5 Standard - Reconstitute the IL-5 Standard with 5.0 mL of Calibrator Diluent RD5-5 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6-35 (*for serum/plasma samples*). This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the appropriate Calibrator Diluent into each 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 (500 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, standards, and controls 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 50 µL of Assay Diluent RD1U to each well. Assay Diluent RD1U may contain a precipitate. Mix well before and during use.

4. Add 200 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. A plate layout is provided to record standards and samples assayed.
   For Cell Culture Supernate/Urine Samples: Incubate for 2 hours at room temperature.
   For Serum/Plasma Samples: Incubate for 2.5 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, 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 by decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 µL of IL-5 Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate/Urine Samples: Incubate for 2 hours at room temperature.
   For Serum/Plasma Samples: Incubate for 2.5 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. 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 polystyrene microplate. Readings made directly at 450 nm without correction may be higher and less accurate.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, controls and Standards as instructed.

2. Add 50 µL Assay Diluent RD1U to each well. Mix RD1U well before and during use.

3. Add 200 µL Standard, control or sample to each well.

Cell Culture Supernate/ Urine Samples — **Serum/Plasma Samples**

Incubate 2 hrs. RT — Incubate 2.5 hrs. RT

4. Aspirate and wash 4 times.

5. Add 200 µL Conjugate to each well.

Cell Culture Supernate/ Urine Samples — **Serum/Plasma Samples**

Incubate 2 hrs. RT — Incubate 2.5 hrs. RT

6. Aspirate and wash 4 times.

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

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.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

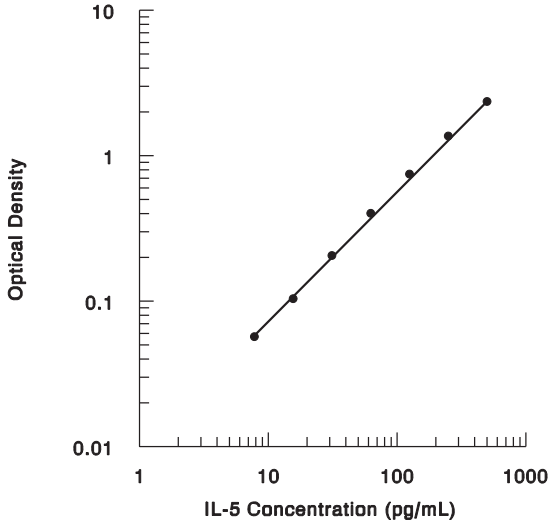
To determine the IL-5 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding IL-5 concentration.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

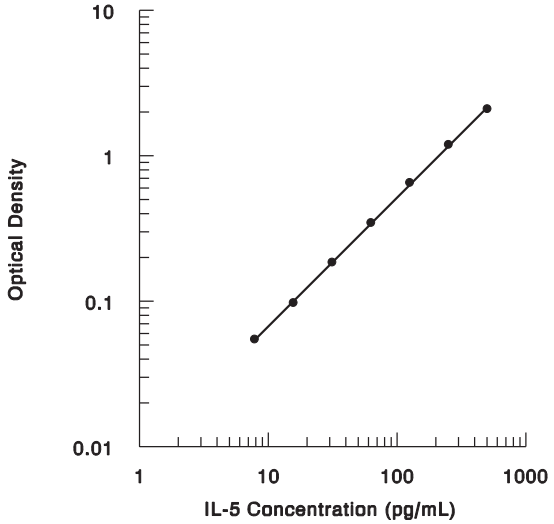
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5-5



(pg/mL)	O.D.	Average	Corrected
0	0.051 0.049	0.050	—
7.8	0.105 0.109	0.107	0.057
15.6	0.155 0.153	0.154	0.104
31.2	0.258 0.254	0.256	0.206
62.5	0.447 0.458	0.452	0.402
125	0.815 0.781	0.798	0.748
250	1.435 1.396	1.416	1.366
500	2.458 2.360	2.409	2.359

Calibrator Diluent RD6-35



(pg/mL)	O.D.	Average	Corrected
0	0.070 0.065	0.068	—
7.8	0.129 0.117	0.123	0.055
15.6	0.163 0.168	0.166	0.098
31.2	0.253 0.254	0.254	0.186
62.5	0.419 0.413	0.416	0.348
125	0.734 0.714	0.724	0.656
250	1.298 1.236	1.267	1.199
500	2.194 2.159	2.176	2.108

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.
- 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.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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.

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)	19.2	159	363	27.0	178	346
Standard deviation	1.1	6.3	10.0	2.7	11.3	17.3
CV (%)	5.7	4.0	2.8	10.0	6.3	5.0

Cell Culture Supernate/Urine Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	20.6	153	363	20.8	134	266
Standard deviation	0.5	2.7	4.7	2.1	7.7	14.0
CV (%)	2.4	1.8	1.3	10.1	5.7	5.3

RECOVERY

The recovery of IL-5 spiked to different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	90 - 105%
Serum (n=7)	99	83 - 108%
EDTA plasma (n=7)	97	85 - 107%
Heparin plasma (n=7)	101	90 - 113%
Citrate plasma (n=7)	98	82 - 110%
Urine (n=7)	98	86 - 104%

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentrations of IL-5 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	Urine
1:2	Average % of Expected	103	103	99	107	104	105
	Range (%)	97 - 107	97 - 105	97 - 100	104 - 110	100 - 109	102 - 107
1:4	Average % of Expected	102	102	98	108	103	106
	Range (%)	98 - 108	96 - 105	95 - 102	105 - 109	98 - 110	101 - 110
1:8	Average % of Expected	91	102	92	108	98	101
	Range (%)	87 - 98	94 - 108	89 - 95	100 - 117	90 - 105	99 - 104
1:16	Average % of Expected	___	100	87	108	102	102
	Range (%)		92 - 104	80 - 93	99 - 115	94 - 116	96 - 104

SENSITIVITY

The minimum detectable dose of IL-5 was typically less than 3.0 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 *Sf* 21-expressed recombinant human IL-5 produced at R&D Systems. The NIBSC/WHO unclassified IL-5 preparation 90/586 (recombinant DNA Human Type) was evaluated in this kit.

The dose response curve of the unclassified standard 90/586 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine IL-5 kit to equivalent NIBSC 90/586 units use the equation below.

NIBSC (90/586) equivalent value (U/mL) = 0.0171 x Quantikine IL-5 value (pg/mL).

SAMPLE VALUES

Serum/Plasma/Urine - Forty serum, plasma, and urine samples were evaluated for the presence of IL-5 in this assay. All samples measured less than the lowest IL-5 standard, 7.8 pg/mL

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum and 50 μM β-mercaptoethanol. Cells were stimulated with 10 μg/mL PHA or 50 ng/mL PMA and 1 μg/mL Ionomycin. The cell culture supernates were removed after 24 hours and assayed for levels of natural IL-5.

Condition	24 hours (pg/mL)
PHA	107
PMA + Ionomycin	227

SPECIFICITY

This assay recognizes both natural and recombinant human IL-5. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent, and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-5 control were assayed for interference. No significant cross-reactivity or interference was observed.

Factors related to or associated with IL-5:

rhGM-CSF rhIL-3 rhIL-5 sRα rhIL-5 sRβ rmIL-5

Other factors:

Recombinant human:
ANG
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
G-CSF
GROα
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-2
IL-4
IL-6
IL-6 sR
IL-7
IL-8
IL-9
IL-10
IL-11
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
OSM
PDGF-AA
PDGF-AB
PDGF-BB
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β2
TGF-β3
TNF-α
TNF-β
sTNF RI
sTNF RII

Recombinant mouse:
GM-CSF
IL-1α
IL-1β
IL-3
IL-4
IL-6
IL-7
IL-9
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant amphibian:
TGF-β5

Recombinant chicken:
TGF-β3

Others:
mouse EGF
bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β2

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.