Quantikine® ELISA

Human IL-5 Immunoassay

Catalog Number D5000B
S5000B
PD5000B

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.

TABLE OF CONTENTS

SECTION PAGE

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INTRODUCTION

Interleukin 5 (IL-5) is a secreted glycoprotein that belongs to the α-helical group of cytokines that includes IL-3, IL-5, and GM-CSF (1-3). Unlike other family members, it is present as an anti-parallel dimer that is covalently linked by two interchain disulfide bonds (4, 5). IL-5 is primarily produced by $CD4^+$ Th2 cells with lower amounts secreted by activated eosinophils and mast cells (1-3, 6). IL-5 increases production and mobilization of eosinophils and $CD34^+$ progenitors from the bone marrow (1, 7). IL-5, produced by Th2 cells and mast cells, is also thought to cause maturation of eosinophil precursors outside the bone marrow (6, 8). A Th2/eosinophil/mast cell axis is thought to be created by eosinophil production of IL-5 and other cytokines and chemokines that amplifies IL-5 production during allergic reactions or parasitic infection (6-9). Production by EBV-transformed B cells, Reed-Sternberg cells in Hodgkin's disease, and IL-2-stimulated invariant natural killer T cells (iNKT) has also been reported (10, 11). Human IL-5 shares 70% amino acid sequence identity with mouse IL-5.

The receptor for human IL-5 consists of a unique ligand-binding subunit (IL-5 Rα) and a common signal-transducing subunit (βc) that is also a component of the IL-3 and GM-CSF receptors (3, 12). Both subunits are members of the cytokine receptor superfamily. IL-5 Rα first binds IL-5 at low affinity and then associates with preformed βc dimers, forming a high-affinity receptor containing at least two of each subunit (13). IL-5 also binds proteoglycans, potentially enhancing its activity (14). In addition to the membrane-anchored IL-5 R α-subunit, cDNAs encoding two soluble isoforms of IL-5 Rα have been isolated from mouse and human cells (15). The soluble IL-5 Rα mRNA is predominant in $CD34^+$ human umbilical cord cells, but the transmembrane form is upregulated upon IL-5 stimulation and eosinophilic differentiation (16, 17). Both forms may be upregulated during eosinophilia (17). Treatment of mature eosinophils with IL-5 causes downregulation of surface IL-5 Rα, which attenuates further responsiveness (18). Soluble IL-5 Rα functions as an IL-5 antagonist *in vitro* (8, 16, 17). IL-5 Rα is mainly expressed by eosinophils but can be found in lower amounts on basophils and mast cells (3).

In humans, IL-5 primarily affects cells of the eosinophilic lineage and promotes their differentiation, maturation, activation, migration, and survival (1-3, 7, 8, 19). Genetic deletion in mice reduces, but does not eliminate, eosinophil numbers or activity presumably due to the actions of IL-3, GM-CSF, and eotaxin, which also promote eosinophil progenitor production, expansion, and migration (7, 20-22). Although eosinophilic infiltration of the lungs is a hallmark of allergic asthma and can be promoted by IL-5, therapeutic anti-IL-5 antibodies did not appear to alter asthma symptoms in clinical trials (23). Anti-IL-5 can, however, reduce circulating eosinophil counts by > 50% and has shown efficacy in hypereosinophilic syndrome (24). In mice, IL-5 enhances Ig class switching and release from B1 cells, but its effect on B cells in humans remains unclear (20, 25). IL-5 is also reported to be a basophil differentiation factor that can prime basophils for histamine and leukotriene release (26, 27).

The Quantikine® Human IL-5 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-5 levels in cell culture supernates, serum, plasma, and urine. It contains *Sf* 21-expressed recombinant human IL-5 and antibodies raised against the recombinant factor and has been shown to accurately quantitate 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 this kit can be used to determine relative mass values for natural human IL-5.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human 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 monoclonal antibody specific for human 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 lots or sources.
- 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 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 # D5000B	CATALOG # S5000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-5 Microplate	893676	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-5.	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 IL-5 Conjugate	893765	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IL-5 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-5 Standard	890201	1 vial	6 vials	Recombinant human IL-5 in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/urine samples.*	
Calibrator Diluent RD6-11	895489	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
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.

D5000B contains sufficient materials to run an ELISA on one 96 well plate.
S5000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD5000B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-5 Controls (optional; R&D Systems®, Catalog # QC20).

PRECAUTIONS

Calibrator Diluent RD6-11 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 preservative 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 MSDS 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 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 heparin as an anticoagulant. Centrifuge for 15 minutes at approximately 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Lipemic, icteric, or hemolyzed samples are not suitable for use in this assay.
High levels of serum albumin may interfere in this assay.

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.

REAGENT PREPARATION

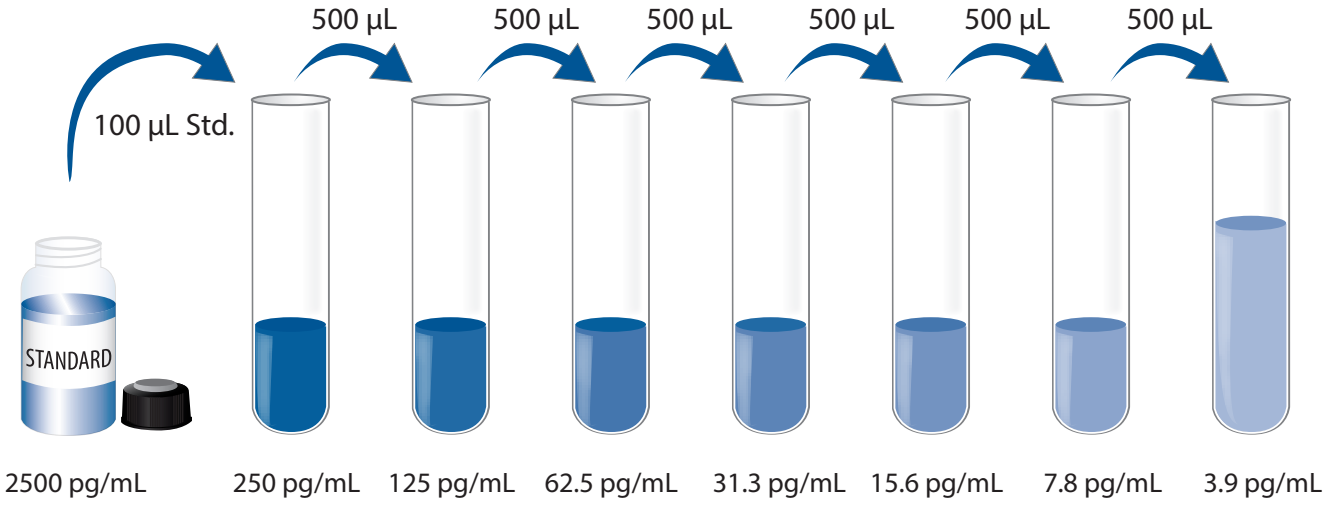
Bring all reagents to room temperature before use.

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 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 IL-5 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-5 Standard with deionized or distilled water. This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-11 (*for serum/plasma samples*) into the 250 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 250 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, samples, 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, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12″ orbit) set at 500 ± 50 rpm.
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 Human IL-5 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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.

CALCULATION OF RESULTS

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

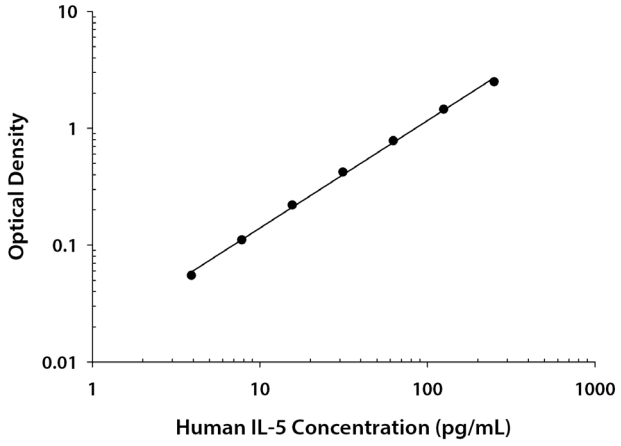
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-5 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

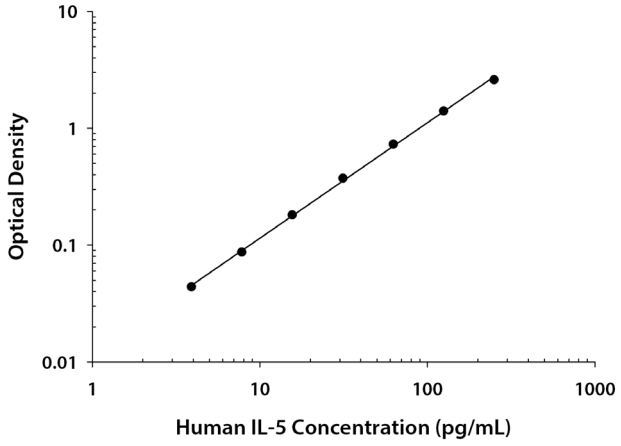
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
3.9	0.063 0.066	0.065	0.055
7.8	0.120 0.121	0.121	0.111
15.6	0.228 0.231	0.230	0.220
31.3	0.429 0.434	0.432	0.422
62.5	0.789 0.795	0.792	0.782
125	1.456 1.461	1.459	1.449
250	2.496 2.521	2.509	2.499

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.013	0.012	—
3.9	0.055 0.056	0.056	0.044
7.8	0.098 0.100	0.099	0.087
15.6	0.190 0.196	0.193	0.181
31.3	0.380 0.389	0.385	0.373
62.5	0.735 0.749	0.742	0.730
125	1.405 1.427	1.416	1.404
250	2.570 2.642	2.606	2.594

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/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	28.1	89.3	170	31.2	92.6	174
Standard deviation	0.89	2.0	7.3	2.5	4.2	8.7
CV (%)	3.2	2.2	4.3	8.0	4.5	5.0

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	31.3	95.6	173	32.9	97.9	180
Standard deviation	1.4	3.5	6.5	4.0	6.7	8.4
CV (%)	4.5	3.7	3.8	12.2	6.8	4.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	95-107%
Serum (n=4)	104	88-116%
EDTA plasma (n=4)	104	86-118%
Heparin plasma (n=4)	101	87-112%
Urine (n=4)	96	91-106%

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of human IL-5 ranged from 0.06-1.08 pg/mL. The mean MDD was 0.29 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 IL-5 were diluted with 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)	Heparin plasma (n=5)	Urine (n=5)
1:2	Average % of Expected	105	103	101	99	102
	Range (%)	102-109	100-105	97-105	86-106	101-103
1:4	Average % of Expected	109	104	102	102	100
	Range (%)	105-112	96-107	96-107	91-107	94-103
1:8	Average % of Expected	107	104	104	100	101
	Range (%)	102-113	96-110	99-107	91-108	99-104
1:16	Average % of Expected	106	105	106	101	94
	Range (%)	97-112	97-117	105-107	90-108	90-99

CALIBRATION

This immunoassay is calibrated against highly purified *Sf* 21-expressed recombinant human IL-5 produced at R&D Systems®.

The NIBSC/WHO Reference Reagent for IL-5 (human; rDNA-derived) 90/586 was evaluated in this kit. The dose response curve of the interim reference material parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® kit to approximate NIBSC (90/586) units, use the equation below.

NIBSC (90/586) approximate value (U/mL) = 0.017 x Quantikine® Human IL-5 value (pg/mL)

SAMPLE VALUES

Serum/Plasma/Urine - Ten serum samples, ten EDTA plasma samples, ten heparin plasma samples, and four urine samples from apparently healthy volunteers were evaluated for the presence of human IL-5 in this assay. All samples measured below than the lowest standard, 3.9 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-5.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	101	18.5
Stimulated	109	697

SPECIFICITY

This assay recognizes 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 recombinant human IL-5 control were assayed for interference. No significant cross-reactivity or interference was observed

Recombinant human:	Other recombinants:	
IL-3	mouse IL-5	equine IL-5
IL-5 Rα	rat IL-5	feline IL-5
IL-5 Rβ	bovine IL-5	porcine IL-5
	canine IL-5	

Recombinant rhesus macaque IL-5 cross-reacts approximately 44% in this assay.

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