

Quantikine[®] ELISA

Human IL-4 Immunoassay

Catalog Number D4050

S4050

PD4050

For the quantitative determination of human Interleukin 4 (IL-4) 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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Manufactured and Distributed by:

USA R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413

TEL: 800 343 7475 612 379 2956

FAX: 612 656 4400

E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.

19 Barton Lane, Abingdon Science Park

Abingdon OX14 3NB, UK

TEL: +44 (0)1235 529449

FAX: +44 (0)1235 533420

E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.

Unit 1901, Tower 3, Raffles City Changning Office,

1193 Changning Road, Shanghai PRC 200051

TEL: +86 (21) 52380373 (400) 821-3475

FAX: +86 (21) 52371001

E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Interleukin 4 (IL-4) is a pleiotropic cytokine produced primarily by activated T lymphocytes, mast cells and basophils (1-3). IL-4 has multiple immune response-modulating functions on a variety of cell types. It is an important regulator of isotype switching, inducing IgE production in B lymphocytes. It is an important modulator of the differentiation of precursor T helper cells to the Th2 subset that mediates humoral immunity and modulates antibody production. In addition, IL-4 has also been shown to have anti-tumor activity both *in vivo* and *in vitro* (1-3).

The sequence of human IL-4 cDNA predicts a 153 amino acid (aa) residue precursor protein containing a 24 aa residue signal peptide that is cleaved to form the mature protein (4). At the amino acid sequence level, mature human IL-4 is approximately 50% identical to mouse IL-4 and there is no species cross-reactivity between the two proteins (1, 2). Human IL-4 also shares approximately 30% amino acid sequence identity with human IL-13 and the two cytokines exhibit overlapping biological activities (5, 6). The gene for IL-4 has been mapped to human chromosome 5q, in close proximity to the genes for IL-3, IL-5, IL-13, and GM-CSF (1, 2).

The biological effects of IL-4 are mediated by specific cell surface receptor complexes. One type of functional IL-4 receptor complex consists of the IL-4-binding subunit (IL-4 R) and a second chain, designated the common γ c chain because it has also been identified as a component of the receptor complexes for IL-2, IL-7, IL-9, and IL-15 (7-9). A second type of functional IL-4 receptor complex, consisting of the IL-4 R and the IL-13 R α , has also been proposed (10, 11). Although IL-4 R does not bind IL-13 directly, it has been shown to complex with the low-affinity IL-13 R α to form the functional high-affinity receptor complex for IL-13 (11, 12). In addition to the membrane-bound form of IL-4 R, a naturally occurring soluble form of IL-4 R has been identified in human and mouse biological fluids and in mouse cell culture supernates (13-15). Soluble IL-4 R has been shown to bind IL-4 with high affinity in solution.

The Quantikine[®] Human IL-4 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-4 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-4 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant human IL-4 accurately. Results obtained using natural human IL-4 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 IL-4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-4 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-4 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 # D4050	CATALOG # S4050	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-4 Microplate	890597	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-4.	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-4 Standard	890592	2 vials	12 vials	Recombinant human IL-4 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Discard after use. Use a fresh standard for each assay.
Human IL-4 Conjugate	890591	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human IL-4 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-32	895253	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. <i>Contains a precipitate. Mix well before and during use.</i>	
Calibrator Diluent RD5L	895028	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. <i>For cell culture supernate samples. Use diluted 1:5 in this assay.</i>	
Calibrator Diluent RD6-9	895423	1 vial	6 vials	21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</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.

D4050 contains sufficient materials to run an ELISA on one 96 well plate.

S4050 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD4050). 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 IL-4 Microplate	890597	50 plates
Human IL-4 Conjugate	890591	50 vials
Human IL-4 Standard	890592	50 vials
Calibrator Diluent RD5L	895028	50 vials
or		
Calibrator Diluent RD6-9	895423	50 vials
Assay Diluent RD1-32	895253	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	750308	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
- 100 mL and 500 mL graduated cylinders
- Test tubes for dilution of standards
- Human IL-4 Controls (optional; R&D Systems®, Catalog # QC01-1)

PRECAUTIONS

Calibrator Diluent RD6-9 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 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, 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.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

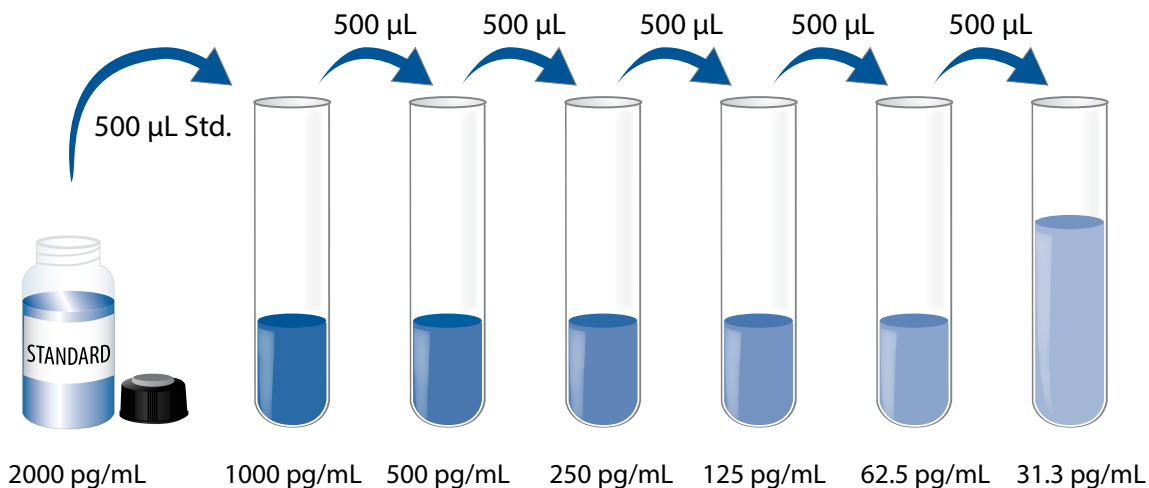
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5L (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5L to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5L (diluted 1:5).

Human IL-4 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human IL-4 Standard with Calibrator Diluent RD5L (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6-9 (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 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 Calibrator Diluent RD5L (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6-9 (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-4 Standard (2000 pg/mL) 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, controls, 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 RD1-32 to each well. *Assay Diluent RD1-32 contains a precipitate. Mix well before and during use.*
4. Add 50 μL of standard, control, or sample per well. Ensure reagent addition is uninterrupted and completed **within 15 minutes**. 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 IL-4 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 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 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.).

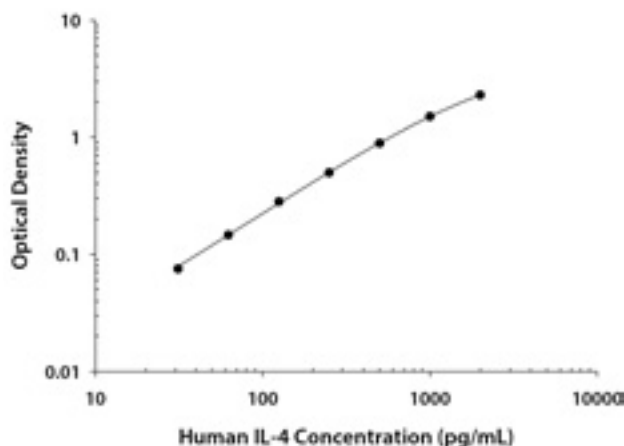
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

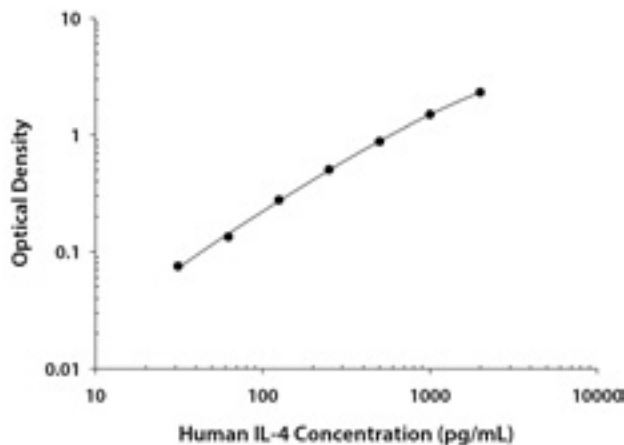
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.033	0.033	—
31.3	0.106 0.110	0.108	0.075
62.5	0.179 0.179	0.179	0.146
125	0.314 0.316	0.315	0.282
250	0.543 0.519	0.531	0.498
500	0.950 0.897	0.923	0.890
1000	1.554 1.513	1.533	1.500
2000	2.317 2.326	2.321	2.288

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.056 0.053	0.054	—
31.3	0.129 0.129	0.129	0.075
62.5	0.192 0.185	0.188	0.134
125	0.331 0.331	0.331	0.277
250	0.561 0.554	0.558	0.504
500	0.938 0.927	0.932	0.878
1000	1.548 1.540	1.544	1.490
2000	2.341 2.380	2.360	2.306

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)	159	538	1092	169	503	1032
Standard deviation	11.4	27.8	67.2	16.8	49.3	83.0
CV (%)	7.2	5.2	6.2	9.9	9.8	8.0

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)	170	510	1110	132	411	865
Standard deviation	6.7	17.5	58.5	9.8	32.1	52.8
CV (%)	3.9	3.4	5.3	7.4	7.8	6.1

RECOVERY

The recovery of human IL-4 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	98	90-109%
Serum (n=10)	98	82-111%
EDTA plasma (n=10)	100	90-123%
Citrate plasma (n=10)	94	85-109%
Heparin plasma (n=10)	95	84-121%

SENSITIVITY

The minimum detectable dose (MDD) of human IL-4 is typically less than 10 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 the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-4 were serially diluted with the appropriate calibrator diluent and then assayed.

		Cell culture media (n=8)	Serum (n=10)	EDTA plasma (n=10)	Heparin plasma (n=10)	Citrate plasma (n=10)
1:2	Average % of Expected	100	99	96	102	102
	Range (%)	94-104	94-104	90-101	96-108	98-110
1:4	Average % of Expected	101	100	95	104	103
	Range (%)	94-116	96-111	89-100	98-110	98-106
1:8	Average % of Expected	95	95	93	102	102
	Range (%)	90-102	90-101	86-100	96-109	95-109
1:16	Average % of Expected	94	92	92	100	99
	Range (%)	82-103	81-100	82-99	84-112	92-108

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-4 produced at R&D Systems®.

The NIBSC/WHO IL-4 1st International Standard 88/656, which was intended as a potency standard, was evaluated in this kit. This standard is an *E. coli*-expressed recombinant human IL-4. The dose response curve of this 1st International Standard parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human IL-4 kit to approximate NIBSC 88/656 units, use the equation below.

NIBSC (88/656) approximate value (IU/mL) = 0.0163 x Quantikine® Human IL-4 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Forty samples from apparently healthy volunteers were evaluated for the presence of human IL-4 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest IL-4 standard, 31.3 pg/mL.

Cell Culture Supernates - Human peripheral blood lymphocytes (PBLs) were isolated from whole blood using ficoll-hypaque and passed through a R&D Systems® T Cell enrichment column. Eluted T Cells were cultured at 2×10^6 /mL in DMEM with 10% fetal bovine serum, 5 µg/mL PHA, and 10 ng/mL recombinant human IL-2. After 6 days of culture, cells were changed into DMEM with 10% fetal bovine serum, 10 ng/mL PMA, 1 µg/mL Ca^{2+} ionophore, and 5 µg/mL PHA. Cell culture supernate was collected after 20 hours of stimulation and measured 782 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-4.

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-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Common γ Chain
GM-CSF
gp130
IL-2 Ra
IL-3
IL-3 Ra
IL-4 Ra
IL-5
IL-5 Ra
IL-6 R
IL-7
IL-13
IL-13 Ra1
IL-13 Ra2

Recombinant mouse:

Common γ Chain
GM-CSF
IL-3
IL-4
IL-4 Ra
IL-5
IL-6
IL-7
IL-13
IL-13 Ra1
IL-13 Ra2

Other recombinants:

bovine IL-4
canine IL-4
cotton rat IL-4
equine IL-4
feline IL-4
porcine IL-4
rabbit IL-4
rat IL-4
rhesus macaque IL-4

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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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