

Quantikine[®] ELISA

Human IL-6 R α Immunoassay

Catalog Number DR600

SR600

PDR600

For the quantitative determination of human Interleukin 6 Receptor alpha (IL-6 R α) concentrations in cell culture 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 6 (IL-6) is a multifunctional cytokine produced by a wide variety of cell types including T cells, monocyte/macrophages, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astroglomas, and glioblastomas. The effects of IL-6 on different cell types are numerous and varied. These activities include: stimulation of B cell differentiation and antibody secretion; action as a co-stimulant with PHA or Con A to increase IL-2 production and IL-2 receptor expression by T cells; enhancement of differentiation of cytotoxic T cells; action as a growth factor for mature thymic or peripheral T cells, myelomas, hybridomas, plasmacytomas, keratinocytes, and mesangial cells; colony-stimulating activity on hematopoietic cells; induction of neuronal cell differentiation; induction of maturation of megakaryocytes; and stimulation of production of acute phase response proteins by hepatocytes. These various activities indicate that IL-6 plays a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. Elevated IL-6 levels have been reported to be associated with a variety of diseases, including autoimmune diseases, mesangial proliferative glomerulonephritis, psoriasis, and malignancies such as plasmacytomas and myelomas. For reviews of the properties and activities of IL-6 see references 1-3.

The biological activities of IL-6 are initiated by binding of the cytokine to a high-affinity receptor complex consisting of two membrane glycoproteins: an 80 kDa component receptor that binds IL-6 with low affinity (IL-6 R α) and a signal-transducing component of 130 kDa (gp130) that does not bind IL-6 by itself, but is required for high-affinity binding of IL-6 by the complex. Both components of the receptor complex, IL-6 R α and gp130, have been cloned, sequenced, and expressed (4-7).

A soluble form of the IL-6 R α with a molecular weight of approximately 50 kDa has been found in the urine of healthy adult humans (8), in culture medium conditioned by the growth of a human myeloma cell line (9), in culture supernates from PHA-stimulated human PBMC and HTLV-1-positive T cell lines (10), and in the serum of HIV-seropositive blood donors (10). This soluble form of the receptor apparently arises from proteolytic cleavage of membrane-bound IL-6 R α . Soluble forms of human and mouse IL-6 R α have also been constructed by insertion of termination codons into the regions of the IL-6 R cDNAs encoding the external portions of the receptors and prior to the transmembrane domains (11, 12). These soluble receptors have been expressed in COS7 and CHO cells (11, 12) and have been shown to bind to IL-6 in solution and to augment the activity of IL-6 as a result of the binding of the IL-6/IL-6 sR α complex to membrane-bound gp130 (11, 12). The regulation *in vivo* of the shedding of the soluble IL-6 R and the function and significance of these soluble receptors in biological fluids is not currently understood. It has been suggested, however, that pathological states involving elevated levels of IL-6 might also be associated with increased production of soluble IL-6 R (10).

The Quantikine Human IL-6 R α Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-6 R α in cell culture supernates, serum, plasma, and urine. It contains recombinant human IL-6 R α produced in Sf 21 cells and antibodies raised against this protein. This kit has been shown to accurately quantitate the recombinant IL-6 R α . Results obtained using natural IL-6 R α 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-6 R α .

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 R α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 R α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-6 R α 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-6 R α 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, further dilute the samples with 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.
- 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 # DR600	CATALOG # SR600	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 Ra Microplate	890114	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-6 Ra.	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.*
Human IL-6 Ra Standard	890116	1 vial	6 vials	10 ng/vial of recombinant human IL-6 Ra in a buffered protein base with preservatives; lyophilized.	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human IL-6 Ra Conjugate	890115	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human IL-6 Ra conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-1	895143	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5C Concentrate	895046	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives.	
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.

DR600 contains sufficient materials to run an ELISA on one 96 well plate.

SR600 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDR600). 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.

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 and samples.
- Human IL-6 Ra Controls (optional; R&D Systems, Catalog # QC05).

PRECAUTIONS

Calibrator Diluent RD5C Concentrate 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 ProClin® 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. Please refer to the MSDS on our website prior to use.

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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.

Note: *Human serum used in the preparation of cell culture media may contain high levels of IL-6 sR. Because of the low species cross-reactivity of this kit, human IL-6 sR levels in culture media containing 10% bovine or fetal bovine serum can be assayed without interference.*

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.

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.

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 μ L of sample + 990 μ L of Calibrator Diluent RD5C (diluted 1:5).*

Urine samples require at least a 2-fold dilution. A suggested 2-fold dilution is 125 μ L of sample + 125 μ L of Calibrator Diluent RD5C (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

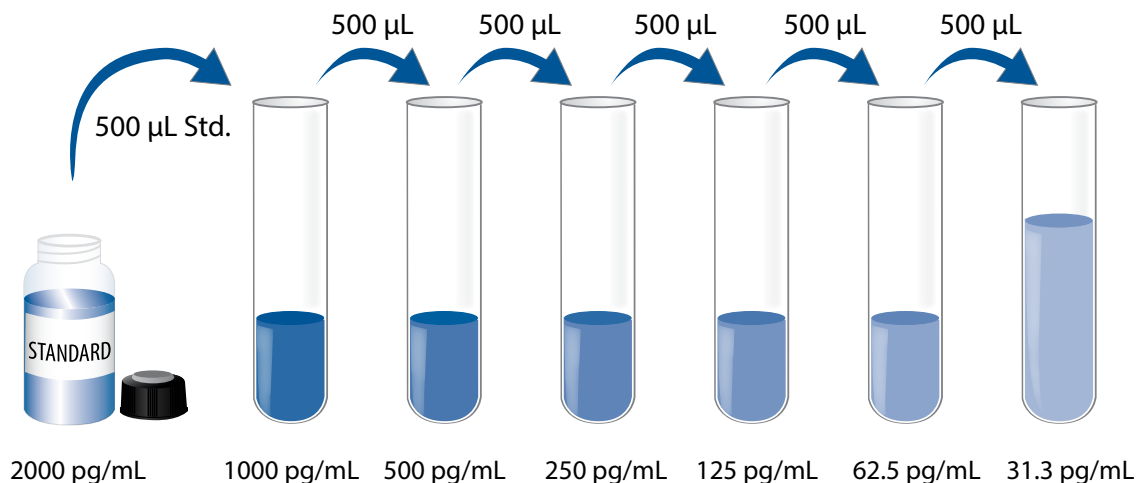
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5C (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5C Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5C (diluted 1:5).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 5 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Human IL-6 R α Standard - Reconstitute the Human IL-6 R α Standard with 5.0 mL of Calibrator Diluent RD5C (diluted 1:5). 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 Calibrator Diluent RD5C (diluted 1:5) 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-6 R α Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5C (diluted 1:5) 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, 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 RD1-1 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. 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-6 R α 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 if 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

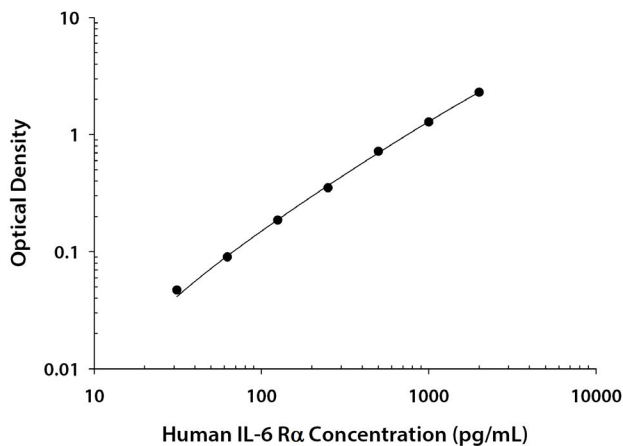
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-6 R α 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.030 0.030	0.030	—
31.3	0.073 0.081	0.077	0.047
62.5	0.115 0.124	0.120	0.090
125	0.214 0.218	0.216	0.186
250	0.377 0.385	0.381	0.351
500	0.732 0.767	0.750	0.720
1000	1.281 1.340	1.311	1.281
2000	2.261 2.394	2.328	2.298

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. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	134	644	1669	130	645	1593
Standard deviation	11.5	16.8	38.3	8.3	26.9	75.4
CV (%)	8.6	2.6	2.3	6.4	4.2	4.7

RECOVERY

The recovery of human IL-6 Ra spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	93	85-97%
Serum*	93	86-110%
EDTA plasma*	93	76-107%
Heparin plasma*	101	87-118%
Citrate plasma*	93	86-102%
Urine*	86	64-98%

*Samples were diluted prior to assay as directed in Sample Preparation.

SENSITIVITY

Forty-five assays were evaluated and the minimum detectable dose (MDD) of human IL-6 Ra ranged from 1.5-15.1 pg/mL. The mean MDD was 6.5 pg/mL.

CALIBRATION

This immunoassay is calibrated against a highly purified Sf 21-expressed recombinant human IL-6 Ra produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-6 Ra were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=8)	EDTA plasma* (n=8)	Heparin plasma* (n=8)	Citrate plasma* (n=8)	Urine* (n=4)
1:2	Average % of Expected	104	101	104	106	106	111
	Range (%)	97-108	79-113	97-110	99-112	98-116	107-115
1:4	Average % of Expected	114	98	105	106	106	118
	Range (%)	111-116	74-109	103-109	97-114	99-114	111-126
1:8	Average % of Expected	118	96	103	106	103	114
	Range (%)	115-120	83-101	92-110	92-112	92-112	106-122
1:16	Average % of Expected	121	98	106	110	111	116
	Range (%)	118-124	93-104	101-111	108-114	103-114	108-124

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SAMPLE VALUES

Serum/Plasma -Samples from apparently healthy volunteers were evaluated for the presence of human IL-6 Ra 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=40)	30,884	13,547-44,942	7548
EDTA plasma (n=40)	30,514	17,114-46,513	7380
Heparin plasma (n=40)	30,459	16,980-47,916	7263
Citrate plasma (n=40)	26,720	14,757-39,888	6458

Cell Culture Supernates - Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were stimulated for 1, 3, and 5 days.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
10 μ g/mL PHA	76	185	348
10 μ g/mL PHA, 10 ng/mL rhIL-2	85	225	463
50 ng/mL PMA	102	166	340
50 ng/mL LPS	69	76	165

Urine - Thirty-six urine samples were evaluated for the presence of IL-6 sR in this assay. The urine IL-6 Ra levels averaged 920 pg/mL and ranged from 132 pg/mL to 1908 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-6 sR.

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-6 sR control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG	IL-8
β -ECGF	IL-9
EGF	IL-10
Epo	IL-11
FGF acidic	LIF
FGF basic	M-CSF
FGF-4	MCP-1
G-CSF	MIP-1 α
GM-CSF	MIP-1 β
gp130	OSM
gp130/Fc Chimera	PDGF-AA
GRO α	PDGF-AB
IFN- γ	PDGF-BB
IGF-I	RANTES
IGF-II	SCF
IL-1 α	SLPI
IL-1 β	TGF- α
IL-1ra	TGF- β 1
IL-2	TGF- β 3
IL-3	TNF- α
IL-4	TNF- β
IL-5	TNF RI
IL-6	TNF RII
IL-7	

Recombinant mouse:

EGF
GM-CSF
gp130/Fc Chimera
IL-1 α
IL-1 β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
MIP-1 α
MIP-1 β
SCF
TNF- α

Other recombinants:

amphibian TGF- β 5
chicken TGF- β 3
cotton rat IL-6
porcine IL-6
rat IL-6

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF- β 1
porcine TGF- β 1
porcine TGF- β 1.2
porcine TGF- β 2

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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