Quantikine® ELISA

Rat IL-6 Immunoassay

Catalog Number R6000B
SR6000B
PR6000B

For the quantitative determination of rat Interleukin 6 (IL-6) 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

Interleukin 6 (IL-6) is a multifunctional cytokine that plays important roles in host defense, acute phase reactions, immune responses, nerve cell functions and hematopoiesis (1-5). It is expressed by a variety of normal and transformed lymphoid and non-lymphoid cells. The production of IL-6 is up-regulated by numerous signals such as mitogenic or antigenic stimulation, lipopolysaccharides, calcium ionophores, cytokines and viruses. IL-4, IL-10 and IL-13 inhibit IL-6 expression in monocytes. Elevated serum IL-6 levels have been observed in a number of pathological conditions, including bacterial and viral infections, trauma, autoimmune diseases, inflammations and malignancies (1-5).

IL-6 is a prototypic member of the IL-6 superfamily of cytokines that share gp130 as a component required for signal transduction (4). The rat, mouse and human IL-6 cDNAs have been cloned (6-9). Rat IL-6 cDNA encodes a 211 amino acid (aa) residue precursor polypeptide with a hydrophobic signal peptide that is cleaved to generate the 187 aa residue mature protein. At the protein sequence level, there is approximately 39% identity between rat and human, and 87% identity between mouse and rat IL-6 (6). Although human and mouse IL-6 are equally active on mouse cells, mouse IL-6 is not active on human cells (9).

The high-affinity IL-6 receptor complex, which mediates IL-6 bioactivity, consists of two membrane glycoproteins: an 80 kDa low-affinity IL-6-binding receptor (IL-6 R) and a 130 kDa signal-transducing protein (gp130) that lacks IL-6 binding ability (4). The binding of IL-6 to IL-6 R recruits gp130 to form a trimeric complex that dimerizes to form a hexameric complex, which transduces IL-6 signal (4). Soluble forms of both IL-6 R and gp130 have been detected in blood (11, 12). Soluble IL-6 R is capable of binding IL-6 and inducing homodimerization of membrane gp130 and subsequent signal transduction.

The Quantikine Rat IL-6 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-6 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant rat IL-6 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant protein. Results obtained using natural rat IL-6 showed dose-response 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 rat IL-6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IL-6 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any rat IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of rat IL-6 bound in the initial step. The sample values are then read off the standard curve.

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 the 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- Allow the plate to soak for at least 30 seconds between washes to improve assay performance.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # R6000B	CATALOG # SR6000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat IL-6 Microplate	890559	1 plate	6 plates	One 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat IL-6.	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.*
Rat IL-6 Standard	890141	2 vials	12 vials	Recombinant rat IL-6 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard within 8 hours of reconstitution. Use a new Standard and Control for each assay.
Rat IL-6 Control	890057	2 vials	12 vials	Recombinant rat IL-6 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Rat IL-6 Conjugate	892704	1 vial	6 vials	12 mL/vial of a polyclonal antibody against rat IL-6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-54	895321	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-16	895302	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives.	
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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid solution.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

R6000B contains sufficient materials to run an ELISA on one 96 well plate.
SR6000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PR6000B). 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.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

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.

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 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 as an anticoagulant. Centrifuge for 20 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 or lipemic samples may not be suitable for use with this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Rat serum samples require a 2-fold dilution into Calibrator Diluent RD5-16 prior to assay.
A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-16. Mix well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

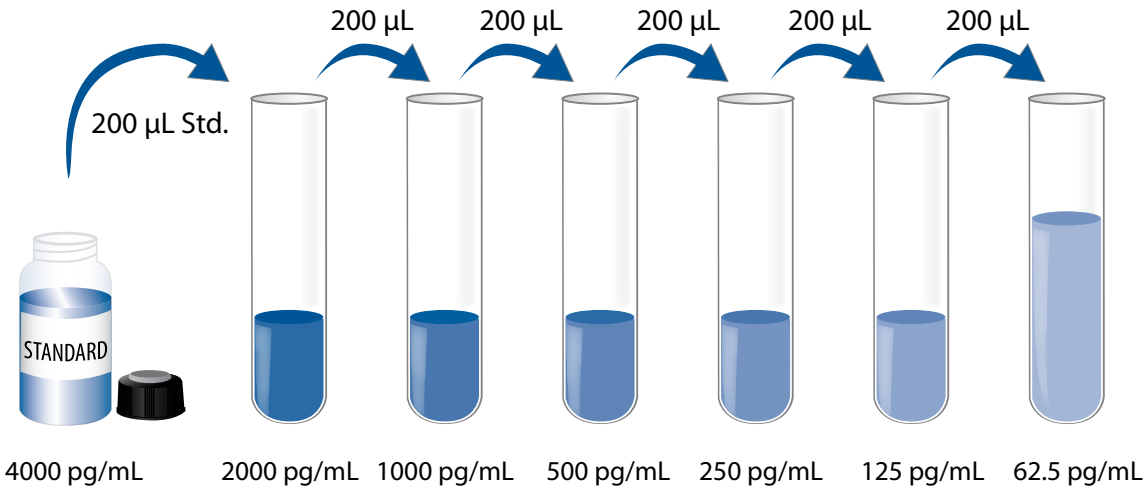
Rat IL-6 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Assay the Control undiluted.

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 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. 100 µL of the resultant mixture is required per well.

Rat IL-6 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat IL-6 Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-16 into each tube. Use the Standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted rat IL-6 Standard serves as the high standard (4000 pg/mL). Calibrator Diluent RD5-16 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 control be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, 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 50 µL of Assay Diluent RD1-54 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Rat IL-6 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

*Serum samples require dilution. See Sample Preparation.

CALCULATION OF RESULTS

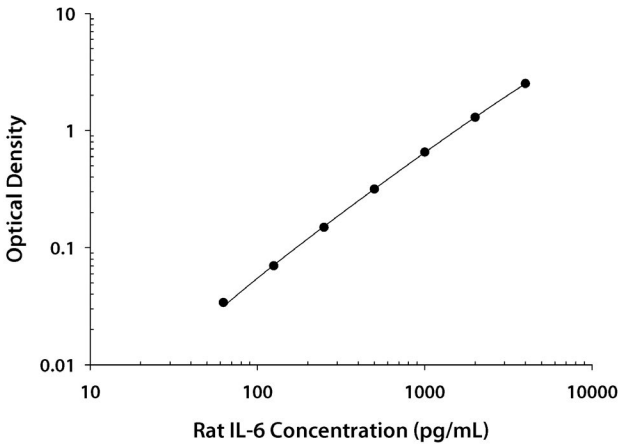
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 rat IL-6 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	0.040 0.052	0.046	—
62.5	0.077 0.082	0.080	0.034
125	0.114 0.117	0.116	0.070
250	0.192 0.198	0.195	0.149
500	0.358 0.365	0.362	0.316
1000	0.670 0.728	0.699	0.653
2000	1.341 1.346	1.344	1.298
4000	2.512 2.603	2.558	2.512

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	22	27	28
Mean (pg/mL)	120	256	1028	134	268	987
Standard deviation	10.6	22.4	46.2	13.4	21.7	69.2
CV (%)	8.8	8.8	4.5	10.0	8.1	7.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	103%	85-118%
Serum* (n=5)	97%	91-105%
EDTA plasma (n=5)	96%	81-111%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of rat IL-6 in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=5)	Serum* (n=5)	EDTA plasma (n=6)
1:2	Average % of Expected	92	100	95
	Range (%)	91-97	98-102	91-101
1:4	Average % of Expected	92	98	99
	Range (%)	88-96	96-101	94-108
1:8	Average % of Expected	88	101	97
	Range (%)	82-93	99-103	85-112
1:16	Average % of Expected	89	101	98
	Range (%)	81-100	96-108	85-115

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

SENSITIVITY

Nine assays were evaluated and the minimum detectable dose (MDD) of rat IL-6 ranged from 14-36 pg/mL. The mean MDD was 21 pg/mL.

The MDD 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 *E. coli*-expressed recombinant rat IL-6 produced at R&D Systems.

SAMPLE VALUES

Serum - Twenty rat serum samples were evaluated for detectable levels of rat IL-6 in this assay. All samples measured less than the lowest rat IL-6 standard, 62.5 pg/mL.

Plasma - Nineteen rat EDTA plasma samples were evaluated for detectable levels of rat IL-6 in this assay. All samples measured less than the lowest standard, 62.5 pg/mL.

Cell Culture Supernates:
Rat spleen cell cultures (1/2 spleen; 1-2 mm pieces in 50 mL DMEM supplemented with 10% fetal bovine serum, containing 50 ng/mL PMA plus 500 ng/mL calcium ionomycin) were collected after culturing for 3 days in a CO_2-enriched (9.5%) incubator. The cell culture supernate was assayed for rat IL-6 and measured 353 pg/mL.

Rat spleen cell cultures (1/2 spleen; 1-2 mm pieces in 50 mL DMEM supplemented with 10% fetal bovine serum, containing 100 ng/mL LPS) were collected after culturing for 3 days in a CO_2-enriched (9.5%) incubator. The cell culture supernate was assayed for rat IL-6 and measured 7033 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat IL-6.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-16 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rat IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

CINC-1
GDNF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-10
IL-18
β-NGF
PDGF-BB
TNF-α

Recombinant human:

sgp130
IL-6
IL-6 sR

Recombinant porcine:

IL-6

Some cross-reactivity was observed with the following:

Recombinant Factor	Concentration (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
Mouse IL-6	100,000	148	0.15

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