Rat IL-6 ELISA Kit

EZRIL6

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Not for use in diagnostic procedures.

USA & Canada Phone: +1(800) 645-5476
In Europe, please contact Customer Service:
France: 0825.045.645;
Spain: 901.516.645 Option 1
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Italy: 848.845.645
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Introduction

Interleukin 6 (IL-6) is a prototypic member of the IL-6 superfamily of cytokines. It is secreted by a variety of lymphoid and non-lymphoid cells including: T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells and keratinocytes. Mature rat IL-6 is a 187 amino acid residue protein. At the protein sequence level, there is 39% identity between rat and human, and 87% identity between rat and mouse. The production of IL-6 can be induced by numerous signals, such as mitogenic or antigenic stimulation, lipopolysaccharides, calcium ionophores, cytokines and viruses.

As a pleiotropic cytokine, IL-6 plays important roles in immune response, inflammation, hematopoiesis, bone metabolism, nerve cell functions and cancer progression. Elevated serum IL-6 levels have been observed in a number of pathological conditions, including bacterial and viral infections, trauma, autoimmune diseases, inflammation and malignancies.

The Rat IL-6 ELISA Kit is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantification of rat IL-6 from cell culture supernatant, serum, plasma and other biological fluids. It is analytically validated with ready-to-use reagents.

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Materials Provided

Store at 2℃ to 8℃		
Component	**Item No.**	**Volume**
Anti-Rat IL-6 pre-coated 96-well plate	CS211400	1 plate
Rat IL-6 Detection Antibody	CS211401	1 bottle (12 mL)
Rat IL-6 Standard	CS211402	1 vial (lyophilized)
Matrix C (for serum and plasma samples)	CS210060	1 vial (lyophilized
Avidin-HRP A	CS210051	1 bottle (12 mL)
Assay Buffer A	CS210062	1 bottle (25 mL)
Wash Buffer (20X)	CS210053	1 bottle (50 mL)
Substrate Solution F	CS210054	1 bottle (12 mL)
Stop Solution	CS213422	1 bottle (12 mL)
Plate Sealers	CS210056	4 sheets

Materials Required But Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 mL to 1,000 mL
- Deionized water
- Wash bottle or automated microplate washer
- Log-log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

Warnings and Precautions

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS for details.
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and plasma in accordance with NCCLS regulations.
- Stop Solution contains acid. *Wear eye, hand, and face protection.*
- Before disposing the plate at end of experiments, rinse the plate with copious amount of tap water.

Storage and Stability

Store at 2℃-8ºC; performance guaranteed for 4 months from date of receipt when reagents are stored properly.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine an optimal dilution factor to ensure accurate quantification.
Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at -70℃. Avoid repeated freeze/thaw cycles.
Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x g. Remove serum layer and assay immediately or store serum samples at < -70℃. Avoid repeated freeze/thaw cycles.
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃. Avoid repeated freeze/thaw cycles.

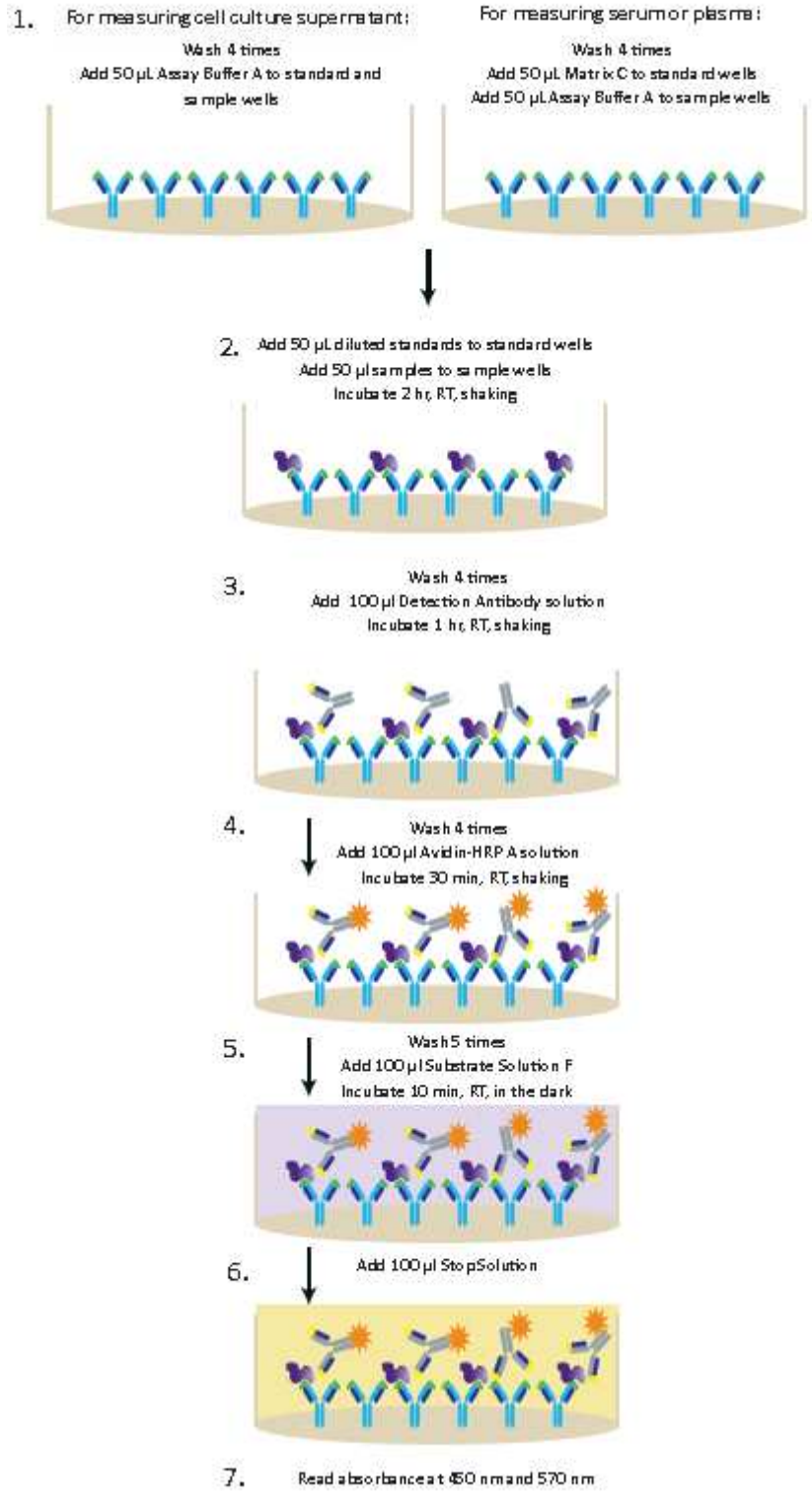
Reagent and Sample Preparation

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
2. Reconstitute the lyophilized Rat IL-6 Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 6 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. If serum or plasma samples will be assayed, reconstitute the lyophilized Matrix C by dispensing 2 mL of deionized water into the vial and allow the reconstituted Matrix C to sit at room temperature for 15 minutes, then vortex to mix completely.
4. In general, cell culture supernatant samples are analyzed without dilutions. However, if dilutions are required, use the control culture medium or Assay Buffer A as the sample diluent.
5. For measuring serum or plasma samples, a 2-fold dilution of the samples with Assay Buffer A is required. If further dilution is necessary, samples should be further diluted with Matrix C.

Protocol

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 300 µL of the 1,200 pg/mL top standard by diluting 60 µL of the standard stock solution in 240 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the rat IL-6 standard concentrations in the tubes are 1,200 pg/mL, 600 pg/mL, 300 pg/mL, 150 pg/mL, 75 pg/mL, 37.5 pg/mL, and 18.8 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).
4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. **For measuring samples of cell culture supernatant:**
    a) Add 50 µL of Assay Buffer A to each well that contains standard dilutions or samples
    b) Add 50 µL of standard dilutions or samples to the appropriate wells.
6. **For measuring serum or plasma samples:**
    a) Add 50 µL of Matrix C to each well that will contain the standard dilutions. Add 50 µL of Assay Buffer A to each well that will contain samples.
    b) Add 50 µL of standard dilutions to the wells containing Matrix C. Add 50 µL of diluted serum or plasma samples to the wells containing Assay Buffer A.
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 µL of Rat IL-6 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing rat IL-6 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

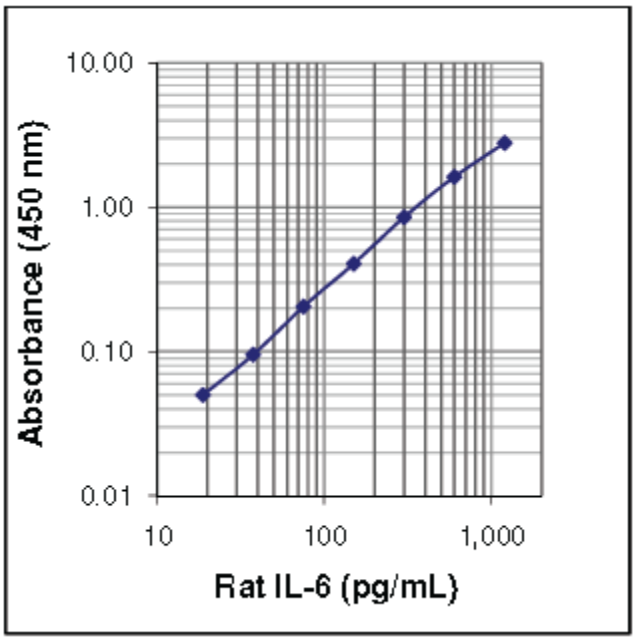
Assay Procedure Summary



Data Analysis

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.
If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:
This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



Product Performance

Specificity: 4.1% cross-reactivity was observed for mouse IL-6. No other cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines/chemokines, each at a concentration of 50 ng/mL.

Human	IL-6
Rat	IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17A, IL-17F, IL-18, IL-22, IL-23, IL-33, CCL20, GM-CSF, IFN-g, KC, MCP-1, RANTES, TNF-a

Sensitivity: The average minimum detectable concentration is 5.3 pg/mL.

Recovery: Three levels of recombinant IL-6 (1000, 250, 62.5 pg/mL) were spiked into four rat serum samples, and then analyzed with Rat IL-6 Kit. On average, 94.9% of the cytokine was recovered from the serum samples.

Linearity: Five rat serum samples were spiked with high concentrations of recombinant rat IL-6, diluted with the appropriate matrix to produce samples with concentrations within the dynamic range and then assayed. Dilution linearity ranged from 91% to 100%. On average, a 96% dilution linearity was observed.

Intra-Assay Precision: Two samples containing different IL-6 concentrations were tested with twelve replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (pg/mL)	64.0	188.5
Standard Deviation	2.6	5.0
% CV	4.1	2.7

Inter-Assay Statistics: Two samples containing different concentrations of IL-6 were tested in six independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	6	6
Mean Concentration (pg.mL)	69.4	199.5
Standard Deviation	5.5	17.7
% CV	7.9	8.9

Biological Samples:

- *Serum* - Normal rat serum samples (n=35) were assayed for basal levels of IL-6. Two samples measured 41 pg/mL and 70 pg/mL. All other sample values were below or close to the lowest standard point, 18.8 pg/mL.
- *Cell Culture Supernatants* – Rat splenocytes ($2x10^6$ cells/mL) were cultured in complete RPMI medium with 1 µg/mL LPS at 37˚C for 1 and 4 days and supernatants were collected and assayed. Rat IL-6 concentrations for day 1 and day 4 samples were 31.5 and 496.5 pg/mL, respectively. IL-6 was not detected in unstimulated samples.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN_3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70℃. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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