



Rat IL-6 ELISA

Cat. No: KB3009

Ver 1,1

**ELISA Set for Accurate Quantitation from Cell
Culture Supernatant, Serum, Plasma, or Other Bodily Fluids**

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

Introduction:

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms. The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with autoantibody production in patients with cardiac myxoma. Since then, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations.

Intended Use:

Rat IL-6 ELISA is specifically designed for the accurate quantitation of Rat IL-6 from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Principle:

Rat IL-6 is an indirect sandwich enzyme immunoassay for the quantitative determination of interleukin-6 in rat serum. The standards, samples and biotinylated antibodies react with IL-6 antibody bound to the solid phase of microtiter plates. This immunological reaction results in formation of a sandwich complex. Following an incubation period unbound components are removed by a washing step. The bound antibodies react specifically with avidin conjugated to horse radish peroxidase (HRP). Following an incubation period, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells turning the solution from blue to yellow.

The optical density (OD) of the solution is directly proportional to the amount of specific IL-6 bound. The standard curve is plotted by using the concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of IL-6 is directly read off from the standard curve.

Materials Provided:

1. Microtitre coated plate (96 wells) – 1 no
2. Recombinant rat IL-6 Standard (lyophilized), 4ng/ml – 1 vial
3. Rat IL-6 Detection Antibody, 25 μ L – 1 vial
4. Concentrated Avidin Horseradish Peroxidase (Av-HRP), 5 μ l - 1 vial
5. 20X Wash Buffer – 15ml
6. 20X Assay Buffer – 2ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes to measure volumes ranging from 50µl to 100µl
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Log-Log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant standard at -20°C. Upon first thaw, aliquot recombinant protein into polypropylene vials and store at -20°C. Do not repeatedly freeze/thaw the recombinant protein standard as loss of activity may result.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles.

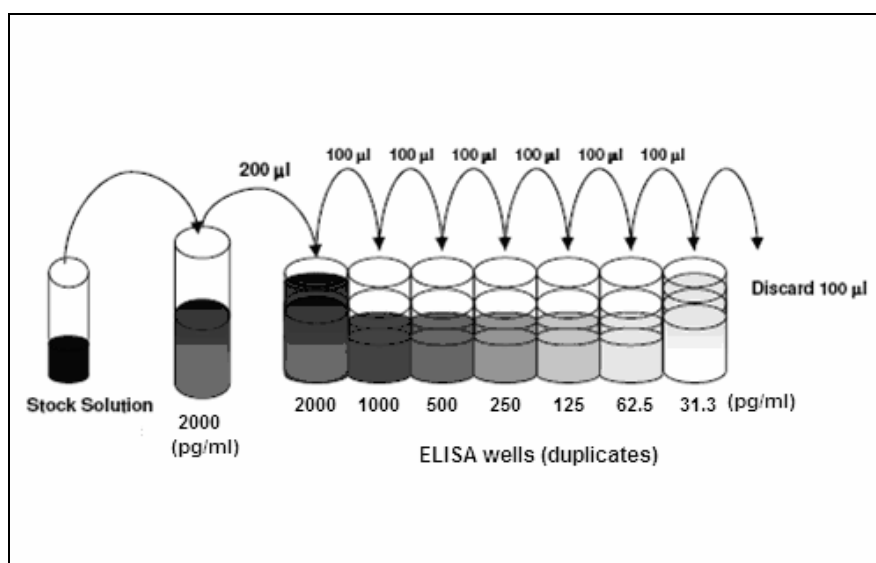
Reagent Preparation (all reagents should be diluted immediately prior to use):

1. 1X Wash Buffer
Dilution: To make 1X Wash Buffer, add 5ml of 20X Wash Buffer to 95ml of DI water. This is the working solution.
2. 1X Assay Buffer
Dilution: To make 1X Assay Buffer, add 2ml of 20X Assay Buffer to 38ml of DI water. This is the working solution.
3. Detection Antibody
Dilution 1:200, add 25 μ l of Detection Antibody solution to 4975 μ l (4.975ml) of 1X Assay Buffer to make final volume to 5ml. Use within 30mins after preparation.*
4. Avidin-HRP
Dilution 1:2000, add 5 μ l of Avidin:HRP solution to 9995 μ l (9.995ml) of 1X Assay Buffer to make final volume to 10ml. Use within 30mins after preparation.*
5. Reconstitute rat IL-6 standard protein with 250 μ l of deionized or distilled water. (final concentration of reconstituted standard = 4ng/ml). Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

**Note: It is recommended due to low volumes quick-spin down the sample for its settlement at the bottom of the vial provided, before dilution.*

Assay Procedure:

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. Add 100 μ l/well of standards and samples to the plate. Perform six two-fold serial dilutions of the 2000 pg/ml top standard, either within the plate or in separate tubes. Thus, the rat IL-6 standard concentrations are 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml. 1X Assay Diluent serves as the zero standard (0pg/ml).



3. Add 50 μ l of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 2 hours.

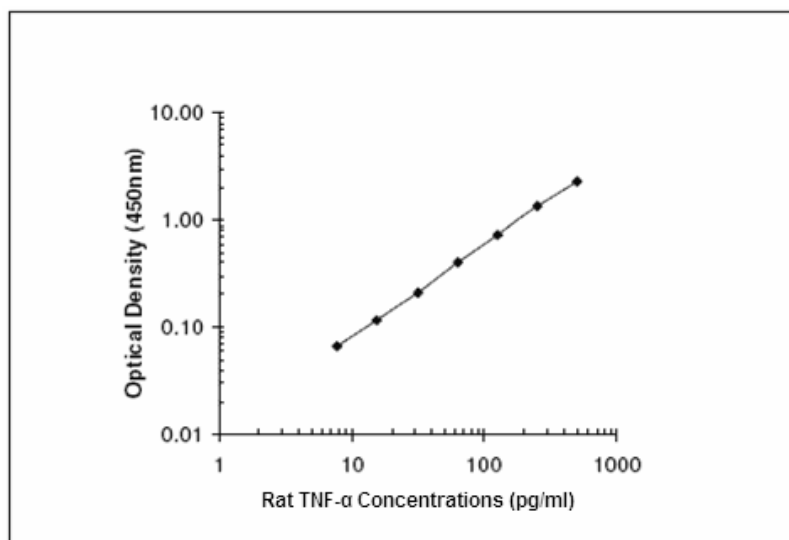
4. Wash plate 4 times with 1X Wash Buffer.
5. Add 100 μ l of diluted Av-HRP solution to each well, seal plate and incubate at RT for 1 hour.
6. Wash plate 4 times with 1X Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
7. Add 100 μ l of TMB Substrate solution and incubate in the dark at RT for 15 minutes. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
8. Stop reaction by adding 100 μ l of Stop Solution to each well. Positive wells should turn from blue to yellow.
9. Read absorbance at 450nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards, controls and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on log-log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns mean absorbance value 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 by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.

**Precautions:**

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Troubleshooting:

Problem	Possible cause	Investigation/Actions
High Absorbances	<ol style="list-style-type: none"> 1. Cross-contamination from other specimens 2. Insufficient or inefficient washing or reading 3. Wavelength of filter not correct. 4. High assay background. 5. Contaminated TMB 6. Incubation time too long or incubation temperature too high. 7. Incorrect dilution of serum 	<ul style="list-style-type: none"> > Repeat assay taking care when washing and pipetting. > Check washer efficiency > Check that the wavelength is 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650 nm. > Repeat assay and include a well that contains only sample diluent or sample absorbent (i.e. a blank well). > Check that TMB is colorless or faint blue. > Check incubation time and temperature. > Check incubator is at the correct temperature. > Repeat assay, ensuring correct serum dilution is used.
Low Absorbances	<ol style="list-style-type: none"> 1. Incubation time too short or incubation temperature too low. 2. Incorrect dilution or pipetting of sera 3. Incorrect filter wavelength. 4. Contaminated Conjugate solution. 5. Kit has expired. 6. Air blank reading high. 7. Incorrect storage of kit. 8. Kit reagents not equilibrated at room temperature 9. Incorrect reagents used. 10. Over washing of plate (e.g. inclusion of a long soak step). 	<ul style="list-style-type: none"> > Ensure time and temperature of assay incubation are correct. > Check incubator is set at the correct temperature. > Repeat assay ensuring correct dilutions and volumes are used. > Ensure controls are sufficiently mixed. > Check the wavelength is set at 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650nm. > Dispense conjugate directly from the bottle using clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugates are clean and free from serum, detergent and bleach. > Check expiration date of kit and do not use if expired. Investigate causes of high background absorbance. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue/purple. > Allow sufficient time for reagents to equilibrate to room temperature prior to assay. > Check the reagents used match those listed on the specification sheet. > Repeat assay using recommended wash procedure.
Poor Duplicates	<ol style="list-style-type: none"> 1. Poor mixing of samples. 2. Poor pipette precision 3. Addition of reagents at inconstant timing intervals; reagent addition takes too long, air bubbles when adding reagents. 4. Inefficient washing - Wash buffer left in wells, inconsistent washing, inadequate washing. 5. Reader not calibrated or warmed up prior to plate reading. 6. Optical pathway not clean 7. Spillage of liquid from wells 8. Serum samples exhibit microbial growth, haemolysis or lipaemia. 9. Uneven well volumes due to evaporation. 	<ul style="list-style-type: none"> > Mix reagents gently and equilibrate to room temperature. > Calibration may need to be checked. > Check pipetting technique-change pipette tip for each sample and ensure excess liquid is wiped from the outside of the tip. > Use consistent timing when adding reagents. > Ensure all dilutions are made before commencing addition to plate. > Improve pipetting technique and skill. > Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing. > Check reader precision > Check reader manual to ascertain warm up time of instrument. > Gently wipe bottom of plate. > Check reader light source and detector are clean. > Repeat assay, taking care not to knock the plate or splash liquid > It is not recommended to use serum samples exhibiting microbial growth, haemolysis or lipaemia. > Cover plate with a lid or plate sealer (not provided).

All wells yellow	<ol style="list-style-type: none"> 1. Contaminated TMB. 2. Contaminated reagents (e.g. Conjugate, Wash buffer). 3. Incorrect dilution of serum. 4. Incorrect storage of kit. 5. Inefficient washing- Wash buffer left in wells, inconsistent washing, inadequate washing. 6. If Conjugate reconstitute is required – Conjugate reconstituted incorrectly. 	<ul style="list-style-type: none"> > Check TMB is colorless or faint blue. > Check reagents for turbidity. > Repeat assay, ensuring correct serum dilution is used. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple. > Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing. > Repeat assay ensuring Conjugate is reconstituted according to assay method.
All wells negative	<ol style="list-style-type: none"> 1. Test not performed correctly – correct reagents not added or not added in the correct sequence. 2. Contaminated Conjugate solution. 3. Over- washing of plate (e.g. inclusion of a long soak step). 4. Incorrect storage of kit. 5. Wash Buffer made up with Stop Solution instead of Wash Buffer Concentrate 	<ul style="list-style-type: none"> > Check procedure and check for unused reagents. > Ensure that Stop Solution was not added before Conjugate or TMB. > Ensure that serum was diluted in correct Sample diluent; e.g. do not use Sample Absorbent for an IgG ELISA. > Dispense Conjugate directly from the bottle using a clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugate are clean and free from serum, detergent and bleach. > Repeat assay using recommended wash procedure. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple. > Ensure Wash Buffer is made up correctly.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Product; against defects in product or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made product or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made product or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the product or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages. This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

*Krishgen BioSystems 2010
Version 1,1*

THANK YOU FOR USING A KRISHGEN PRODUCT !