






Human IL-8 ELISA

REF: KB1070

Ver 5.0

RUO

ELISA for Accurate Quantitation of Human IL-8 from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

For Research Purposes Only. 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:

IL-8 is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. IL-8 plays a role in host defense, acute phase reactions, immune response, and hematopoiesis. IL-8 is expressed by T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells, and keratinocytes. Recombinant human IL-8 is a 20.9KD protein containing 184 amino acids.

Intended Use:

Human IL-8 ELISA is specifically designed for the accurate quantitation of human IL-8 from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Materials Provided:

1. Microtiter Coated Plate (12X8 wells) – 1 no.
2. Recombinant Human IL-8 Standard – 2 vials
3. Human IL-8 Biotin Conjugated Detection Antibody – 1 vial
4. Concentrated Streptavidin Horseradish Peroxidase - 1 vial
5. Wash Buffer (20X) – 25ml
6. Assay Diluent – 50ml
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 1000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-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 thawing, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
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 temperature $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at $1000 \times g$. Remove serum layer and assay immediately or store serum samples at temperature $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

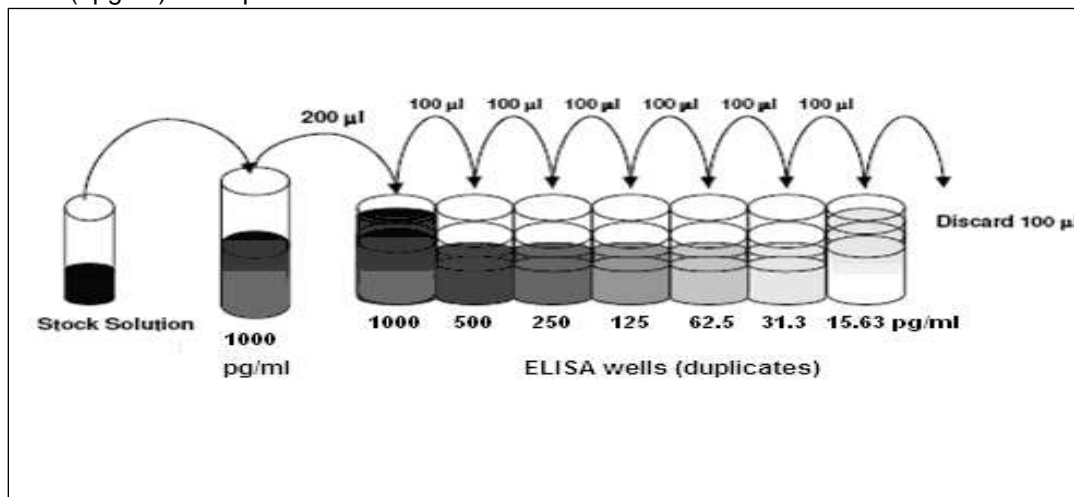
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at $1000 \times g$ within 30 minutes of collection. Assay immediately or store plasma samples at temperature $< -20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

Please refer to lot specific instructions for preparation of the reagents.

Assay Procedure: ALL STEPS TO BE PERFORMED AT 37°C .

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 $50\mu\text{l}$ of diluted Biotin Conjugated **Detection Antibody** solution to each well followed by addition of $100\mu\text{l}$ /well of **Standards** and **Samples** to the plate. Perform two-fold serial dilutions of the 1000pg/ml top standard, either within the plate or in separate tubes. Thus, the Human IL-8 standard concentrations are 1000pg/ml , 500pg/ml , 250pg/ml , 125pg/ml , 62.5pg/ml , 31.3pg/ml and 15.6pg/ml . **Assay Diluent** serves as the zero standard (0pg/ml). Seal plate and incubate for 2 hours at 37°C .



3. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4. Add 100µl of diluted **Streptavidin-HRP** solution to each well, Seal plate and incubate for 30 minutes at 37°C.
5. Wash plate 4 times with **Wash Buffer (1X)** as in step 3. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
6. Add 100µl of **TMB Substrate** solution and incubate in the dark for 30 minutes at 37°C. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
7. Stop reaction by adding 100µl of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
8. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on Semi-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.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is for in vitro use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



LIMITED WARRANTY

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