Humam IL8 ELISA Kit

Enzyme Immunoassay for the quantification of human Interleukin 8 (IL8) in human plasma.

Catalog number: ARG80506

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

Biological activities

IL-8 (also known as NAP-1 for Neutrophil-activating peptide) is a chemoattractant protein for neutrophils. This cytokine belongs to a new family of chemotactic peptides called "chemokines". This proinflammatory mediator is secreted by different cells such as monocytes, neutrophils, endothelial cells, fibroblast after activation, and by mitogen-stimulated T lymphocytes. IL-8 is a key cytokine that has been found in scales of psoriasis patients, in synovial fluid of patients suffering from rheumatoid arthritis and gout. The role of IL-8 in the recruitment of neutrophils in the lung during ARDS has also been suggested.

Clinical application

The IL-8 level in the septic shock patients was found to correlate with mortality and in acute graft liver rejection the IL-8 serum levels were reported to have markedly increased. The level of IL-8 in these or other conditions may prove to be important in characterizing the progress of these disease conditions.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IL-8 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-conjugated antibody specific for IL-8 is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate

solution (TMB) is added to the wells and color develops in proportion to the amount of IL-8 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of IL-8 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard 0-5 (Concentration indicated on vial label)	6 vials (Lyophilized)	4°C.
Controls	2 vials (Lyophilized)	4°C
Specimen diluent	2 vials (Lyophilized)	4°C
Incubation buffer	11ml (Ready-to-use)	4°C
HRP-Antibody conjugate	6ml (Ready-to-use)	4°C
200X Wash buffer	10ml	4°C
TMB substrate	1 vial (1ml)	4°C (Protect from light)
Substrate buffer	3 vials (21ml)	4°C (Protect from light)
STOP solution	6ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 200X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA 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. Hemolytic sample should be avoided.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 100X Wash buffer into distilled water to yield 1X Wash buffer.
- Controls: Reconstitute controls with 1ml distilled water.
- Specimen Diluent: Reconstitute specimen diluent with distilled water as according to the vial label.
- TMB Substrate: Add 0.2ml TMB substrate into 1 vial of substrate buffer. (Prepare 15 minutes before use)
- Standards: Reconstitute standards with 1ml distilled water.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of incubation buffer into all wells.
3. Add 100 µl of standards, controls, samples and zero controls into wells.
4. Add 50 µl 1X Antibody solution into each well. Incubate for 2h at RT.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 200 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature.
7. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

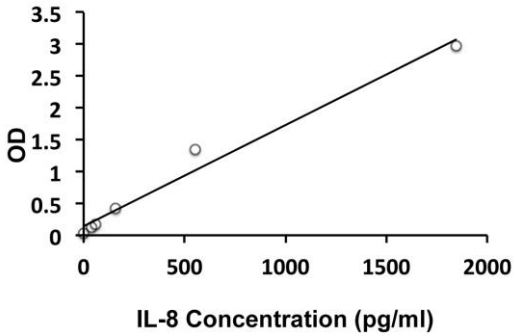
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of IL-8 ranged from 40.2-1845 pg/ml. The mean MDD was 1.1 pg/ml.

Specificity

No significant cross-reactivity or interference with the following factors was observed:

IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-10, TNF-α, TNF-β, IFN-β, IFN-γ, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, RANTES, PF-4, βTG, GRO, IP-10, SCF.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.3% and inter-assay precision was 10.85%.

Recovery

105-119%