> IL-8 (human), ELISA kit

Catalog # ADI-902-156

10x96 Well Enzyme Immunoassay Kit
For use with plasma, serum and tissue culture supernatants

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Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

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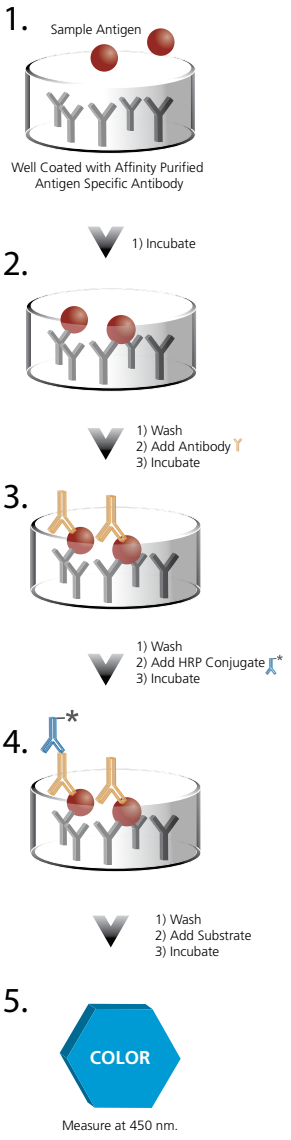
Introduction



Interleukin-8 (IL-8) is a chemokine, a member of the cytokine family that displays chemotactic activity for specific types of leukocytes. IL-8 is a CXC chemokine in which an amino acid is present between the first two of four highly conserved cysteine residues. Chemokines are basic proteins with low molecular weights and an affinity for heparin. The human IL-8 cDNA sequence encodes for a protein of 99 amino acids that undergoes post-translational processing to yield a protein with
77 amino acids and a variant form with 72 amino acids. IL-8 binds to the Duffy antigen on red blood cells and the CXCR1 and CXCR2 G protein coupled receptors (1). Human IL-8 functionality is homologous with mouse, guinea pig, rat and rabbit neutrophils. IL-8 is present in the synovial fluids of patients with rheumatoid arthritis and has been purified from plaque psoriasis scales (2). It is associated also with sepsis, asthma and glomerulonepheritis (3-4). The cytokine will be clinically important in understanding disease states that are characterized by neutrophil infiltration.

IL-8 regulates leukocyte recruitment and trafficking and also has mitogenic and angiogenic properties (5). Monocytes, macrophages, neutrophils, lymphocytes, dermal fibroblasts, keratinocytes, vascular endothelial cells, melanocytes, hepatocytes and various tumor cell lines produce IL-8. IL-8 expression has been demonstrated in neuroendocrine tumor cells in human cancer tissue (6). Serum IL-8 levels increase with the progression of prostate cancer (7,8). The prostate cancer cell line PC3 secretes IL-8 and expresses the CXCR1 and CXCR2 receptors (9). The prostate cancer cell line LNCaP does not secrete IL-8. Selection in low androgen medium created a cell line that produces IL-8 and is more tumorigenic than the parental cell line (10). IL-8 may be important in the transition of prostate cancer to an androgen-independent state (11). Nystatin induces IL-8 secretion in 293 HEK cells through the activation of toll like receptors 1 and 2 (12). IL-8 expression increases angiogenic activity by inducing matrix metalloproteinase type 9 that regulates tumorigenicity and metastasis in human bladder cancer cell line 253J B-V (13). The IL-8 (human), ELISA kit is a complete kit for the quantitative determination of human IL-8 in biological fluids.

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody specific for human IL-8. The plate is then incubated.

2. The plate is washed, leaving only bound human IL-8 on the plate. A yellow solution of polyclonal antibody to human IL-8 is then added. This binds the human IL-8 captured on the plate. The plate is then incubated.

3. The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the human IL-8 polyclonal. The plate is again incubated.

4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. The substrate generates a blue color when catalyzed by the HRP.

5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of human IL-8 in the sample

Materials Supplied

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the molecule.

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

Protect substrate from prolonged exposure to light.

Stop solution is caustic. Keep tightly capped.

1. Assay Buffer 13 Concentrate
   60 mL, Catalog No. 80-2022
   Tris buffered saline containing detergents.

2. human IL-8 Standard
   Catalog No. 80-0660
   Lypholized vials (12) each containing 2,000 pg of recombinant human IL-8.

3. human IL-8 Clear Microtiter Plate
   Ten Plates of 96 Wells, Catalog No. 80-1616
   Clear plates of break-apart strips coated with a mouse monoclonal antibody specific to human IL-8.

4. Wash Buffer Concentrate
   2x250 mL, Catalog No. 80-1799
   Two bottles of tris buffered saline containing detergents.

5. IL-8 (human) ELISA Antibody
   100 mL, Catalog No. 80-2025
   A yellow solution of polyclonal antibody to human IL-8.

6. IL-8 (human) ELISA Conjugate
   100 mL, Catalog No. 80-2026
   A blue solution of donkey anti-rabbit IgG conjugated to horseradish peroxidase.

7. TMB Substrate
   100 mL, Catalog No. 80-2035
   A solution of 3,3’,5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide.

8. Stop Solution 2
   100 mL, Catalog No. 80-2036
   A 1N solution of hydrochloric acid in water.

9. human IL-8 Assay Layout Sheet
   1 each, Catalog No. 30-0236

10. Plate Sealer
    10 each, Catalog No. 30-0012

Storage

All components of this kit, except the human IL-8 Standard, are stable at 4°C until the kit's expiration date. The human IL-8 Standard must be stored at -20°C .

All reagents, except standard, should be stored at 4˚C. Store standard at -20˚C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 50 µL and 1,000 µL.
3. Repeater pipet for dispensing 100 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Lint-free paper for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Graph paper for plotting the standard curve.
10. Disposable polypropylene tubes for dilution of samples and standards.

Reagent Preparation

Bring all reagents to room temperature for at least 30 minutes prior to opening. Except for the standard.

1. Wash Buffer

   Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Assay Buffer

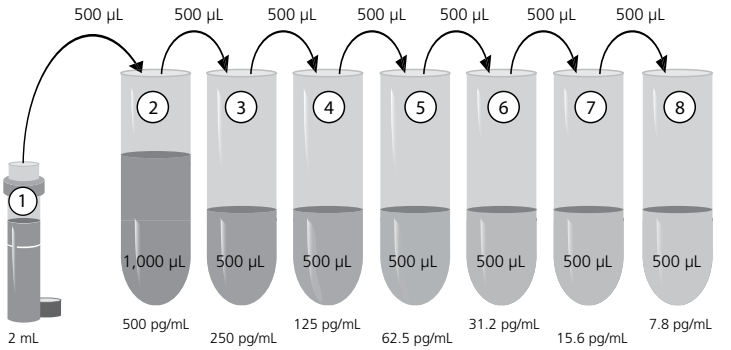
   Just before use, prepare the amount of assay buffer needed by making a 1:10 dilution. As an example, dilute 10 mL of the supplied Assay Buffer 13 Concentrate with 90 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

Plastic tubes must be used for standard preparation.

3. human IL-8 Standards

   Reconstitute one vial of human IL-8 Standard with 2 mL standard diluent (Assay Buffer 13 or tissue culture media). Mix thoroughly without foaming. Label vial of reconstituted human IL-8 Standard #1. Label seven 12x75 mm polypropylene tubes #2 through #8. Pipet 500 µL of standard diluent (Assay Buffer 13 or tissue culture media) into tubes #2 through #8. Add 500 µL of Standard #1 to tube #2. Vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex throughly. Continue this for tubes #4 though #8.

   The concentration of human IL-8 in tubes #1 through #8 will be 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.8 pg/mL respectivly. See human IL-8 Assay Layout for dilution details. STORE STANDARD AT -20°C, avoid repeated freeze-thaws.



Diluted standards should be used within 30 minutes of preparation.
The concentration of human IL-8 in tubes is labeled above. Polypropylene tubes are recommended for diluting the human IL-8 Standards. Alternately, glass tubes may be used, provided the standards are used within 30 minutes of preparation.

Sample Handling

The IL-8 (human), ELISA kit is compatible with human IL-8 samples in a wide range of matrices. Samples can be read directly from a standard curve. Please refer to the Sample Recovery recommendations for details of suggested dilutions. We recommend using polypropylene tubes for sample preparation and storage.

Culture fluids, serum and plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer 13. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of human IL-8 in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive human IL-8. If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at -70 °C to avoid loss of bioactive human IL-8. Up to three freeze/thaw cycles of serum has been shown to have no effect on human IL-8 levels. Nonetheless, excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37ºC incubator. Do not vortex or sharply agitate samples.

Sample Recoveries

Human IL-8 concentrations were measured in a variety of different samples including human serum, plasma and tissue culture medium. Human IL-8 was spiked into the un-diluted samples of these matrices, diluted, and assayed in the kit. The following results were obtained:

Sample	Recovery (%)	Recommended Dilution
Tissue Culture Medium	101	None
Human Serum	98	1:2
Human EDTA plasma	98	1:2

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls, and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 µL of the standard diluent into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #8 to the bottom of the appropriate wells.
3. Pipet 100 µL of the samples to the bottom of the appropriate wells.
4. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
5. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 µL of yellow antibody into each well except the blank.
7. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
8. Wash as above (Step 5).
9. Add 100 µL of blue conjugate to each well except the blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
11. Wash as above (Step 5).
12. Pipet 100 µL of substrate solution into each well.
13. Incubate for 15 minutes on a plate shaker (~500 rpm) at room temperature.
14. Pipet 100 µL of stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of human IL-8 in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the average Net OD for each standard versus human IL-8 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

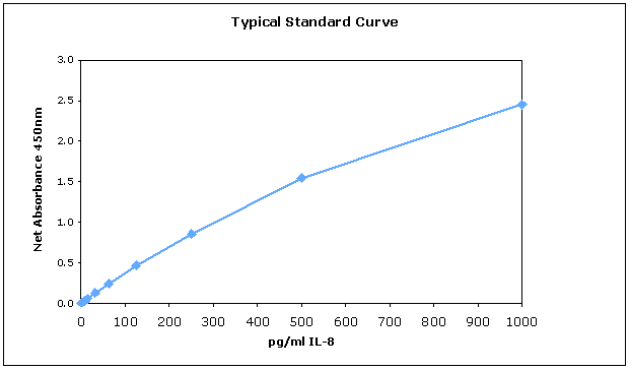
Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	human IL-8 (pg/mL)
S0	0.001	0
S1	2.454	1,000
S2	1.543	500
S3	0.856	250
S4	0.462	125
S5	0.236	62.5
S6	0.125	31.2
S7	0.061	15.6
S8	0.030	7.8
Unknown 1	0.998	294
Unknown 2	0.565	156



Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting potential cross reactants in Assay Buffer 13 at 100,000 pg/mL. These samples were then measured in the assay.

Compound	Cross Reactivity
human IL-2	< .02%
human IL-4	< .02%
human IL-6	< .02%
human IL-7	< .02%
human IL-10	< .02%
GM-CSF	< .02%
TNFα	< .02%

Sensitivity

The sensitivity of the assay, defined as the concentration of human IL-8, measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 0.64 pg/mL.

Linearity

A buffer sample containing hIL-8 was serially diluted and measured in the assay.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1:2	331.6 pg/mL	345.9 pg/mL	104.3 %
1:4	165.8 pg/mL	176.7 pg/mL	106.6 %
1:8	82.9 pg/mL	87.5 pg/mL	105.5 %
1:16	41.5 pg/mL	44.1 pg/mL	106.4 %
1:32	20.7 pg/mL	22.4 pg/mL	108.1 %
1:64	10.4 pg/mL	10.9 pg/mL	105.5 %

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing human IL-8 in a single assay.

pg/mL	%CV
78	7.7
160	3.3
343	2.2

Inter-assay precision was determined by measuring buffer controls of varying human IL-8 concentrations in multiple assays over several days.

pg/mL	%CV
82	8.2
161	6.1
316	7.1

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Notes

Notes

MSDS (Material Safety Data Sheet) available online

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