Product Manual

IL-8 (human) ELISA kit

Catalog #: ADI-900-156A

1x96 Wells

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Product Manual

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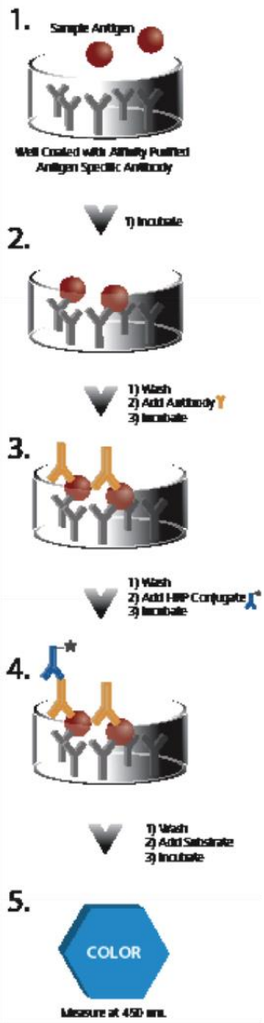
Please read entire booklet before proceeding with the assay.

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 glomerulonephritis (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

This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at -20°C in the sealed bag provided. The wells should be used in the frame provided.

1. **IL-8 Microtiter Plate, One Plate of 96 Wells**
   Catalog No. 80-2882

   A plate using break-apart strips coated with a monoclonal antibody to IL-8.

2. **Assay Buffer 13, 50 mL**
   Catalog No. 80-1500

   Tris buffered saline containing detergents.

3. **Human IL-8 Conjugate, 10 mL**
   Catalog No. 80-2886

   A blue solution of donkey anti-rabbit IgG conjugated to horseradish peroxidase.

4. **Human IL-8 Antibody, 10mL**
   Catalog No. 80-2883

   A yellow solution of polyclonal antibody to human IL-8.

5. **Human IL-8 Standard**
   Catalog No. 80-0660

   Lyophilized vials (2) containing 2,000 pg of recombinant human IL-8.

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

6. **Wash Buffer Concentrate, 100 mL**
   Catalog No. 80-1287

   One bottle containing 20x Tris buffered saline with detergent.

Protect substrate from prolonged exposure to light.

7. **TMB Substrate, 10 mL**
   Catalog No. 80-0350

   A solution of 3,3'5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.

Stop solution is caustic. Keep tightly capped.

8. **Stop Solution 2, 10 mL**
   Catalog No. 80-0377

   A 1N solution of hydrochloric acid in water.

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

10. **Plate Sealer, 3 each**
    Catalog No. 30-0012

STORAGE AND STABILITY

All of the components of this kit, except for 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 and is stable at that temperature until the kit's expiration date.

OTHER MATERIALS NEEDED

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. Benchtop centrifuge
6. Graduated cylinders
7. A microplate shaker
8. Adsorbent paper for blotting
9. Microplate reader capable of reading a 450 nm
10. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

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.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Human serum, EDTA plasma and tissue culture media have been validated 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 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 at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

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 MATRIX PROPERTIES

Linearity

Human EDTA plasma and serum were diluted 1:2 in assay buffer, spiked with IL-8, serially diluted in assay buffer and run in the assay. Additionally, neat tissue culture media was spiked with IL-8, serially diluted in assay buffer and run in the assay. Matrix background was subtracted and the dilutional linearity was determined in both matrices. The data was compared to the standard curve. The results are shown in the table below.

Dilutional Linearity, %			
Dilution	**EDTA Plasma**	**Serum**	**Tissue Culture Media**
Neat	---	---	100
1:2	100	100	90
1:4	104	104	84
1:8	103	106	93
1:16	101	104	81
1:32	105	111	---
1:64	97	110	---
1:128	85	150	---
1:256	---	108	---

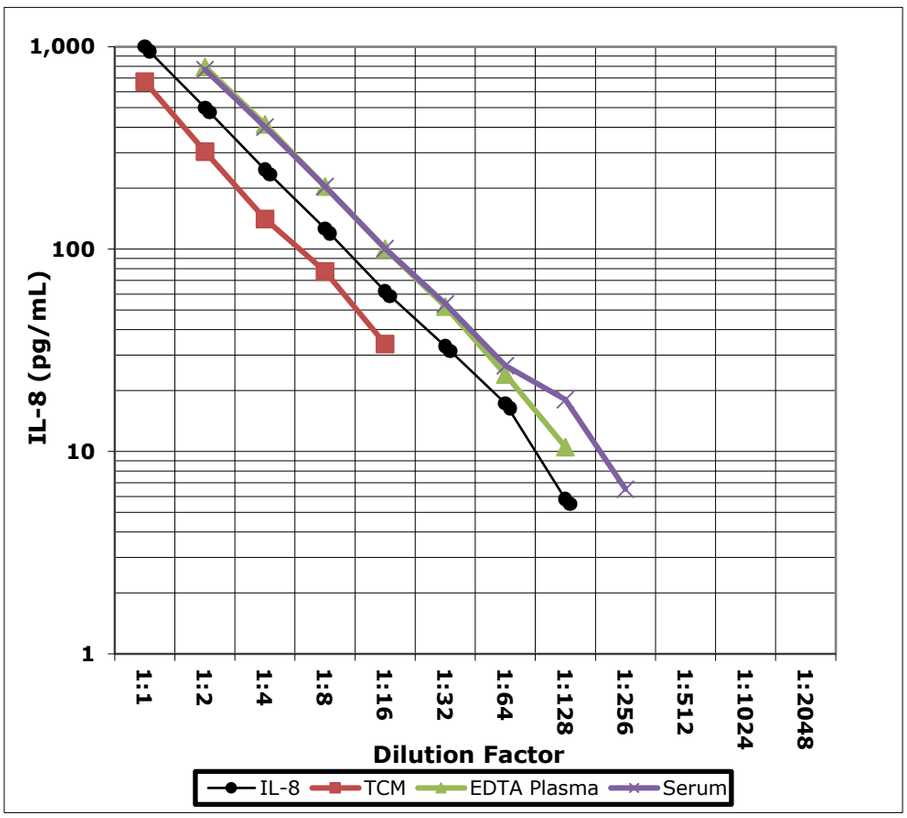
Spike and Recovery

Human IL-8 concentrations were measured in human serum, EDTA plasma and tissue culture medium. Human IL-8 was spiked into 1:2 diluted human serum and plasma and assayed in the kit. Additionally, IL-8 was spiked into neat tissue culture media and assayed in the kit. The following results were obtained:

Sample	Spike Concentration, pg/mL	% Recovery	Minimum Recommended Dilution
EDTA Plasma	400	99	1:2
	100	98	
	25	91	
Serum	400	97	1:2
	100	97	
	25	84	
Tissue Culture Media	400	103	Neat
	100	90	
	25	104	

Parallelism

To assess parallelism, human EDTA plasma and serum were diluted to the MRD of 1:2, spiked with human IL-8, serially diluted in assay buffer and then run in the assay. Additionally, human IL-8 was spiked into neat tissue culture media, serially diluted in assay buffer and run in the assay. The IL-8 concentration in each sample was assigned using the standard curve. Assigned concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human origin.



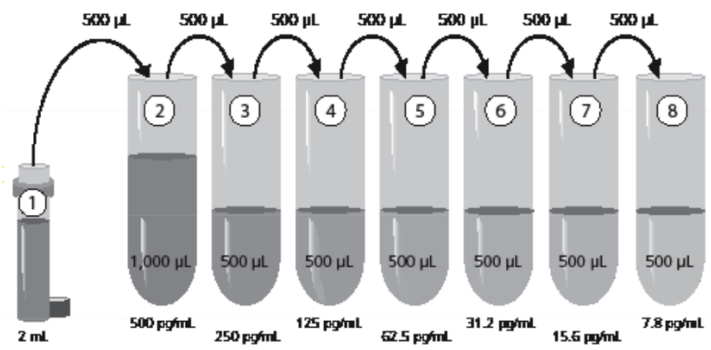
REAGENT PREPARATION

1. **Wash Buffer**

   Prepare Wash buffer by diluting 30 mL of the supplied Wash Buffer concentrate with 570 mL of deionized water. The diluted wash buffer can be stored at room temperature for up to 3 months.

2. **IL-8 Standard**

   Reconstitute one vial of human IL-8 Standard with 2mL 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. Add 500 µL of standard diluent (Assay Buffer 13 or tissue culture media) into tubes #2 through #8. Add 500 µL of Standard #1 into tube #2 and vortex to mix. Add 500 µL of tube #2 into tube #3 and vortex to mix. Continue this for tubes #4 through #8.



Diluted standards should be used within 60 minutes of preparation. Store standard stock at -20°C and avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C.

All standards and samples should be run in duplicate.

1. Add 100 µL of standard diluent (Assay Buffer 13 or tissue culture media) into the S0 (0 pg/mL standard) and NSB (non-specific binding) wells. Leave the Blank wells empty.
2. Add 100 µL of standards #1 through #8 into the appropriate wells.
3. Add 100 µL of the samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature (RT) on a plate shaker for 1 hour at ~500 rpm*****. **See note.**

Pipet the reagent to the side of the wells to avoid possible contamination.

5. Empty the contents of the wells and wash by adding a full well volume (~400 µL) of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 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. Add 100 µL of IL-8 Antibody into each well, except the NSB and Blank. Add 100 µL Assay Buffer 13 into NSB wells and leave the Blank wells empty.
7. Seal the plate. Incubate at RT on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µL of IL-8 Conjugate to each well, except the Blank wells.
10. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5).
12. Add 100 µL of TMB substrate into all wells.
13. Seal the plate and incubate at RT on a plate shaker for 15 minutes at ~500 rpm.
14. Add 100 µL of Stop Solution into each well.
15. After zeroing the plate reader against the Blank, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** Note: The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

CALCULATION OF RESULTS

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

Several options are available for the calculation of the concentration of IL-8 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. The concentration of IL-8 can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus IL-8 concentration in each standard. Samples with concentrations outside of the standard curve range will need to be reanalyzed using alternative dilution(s).

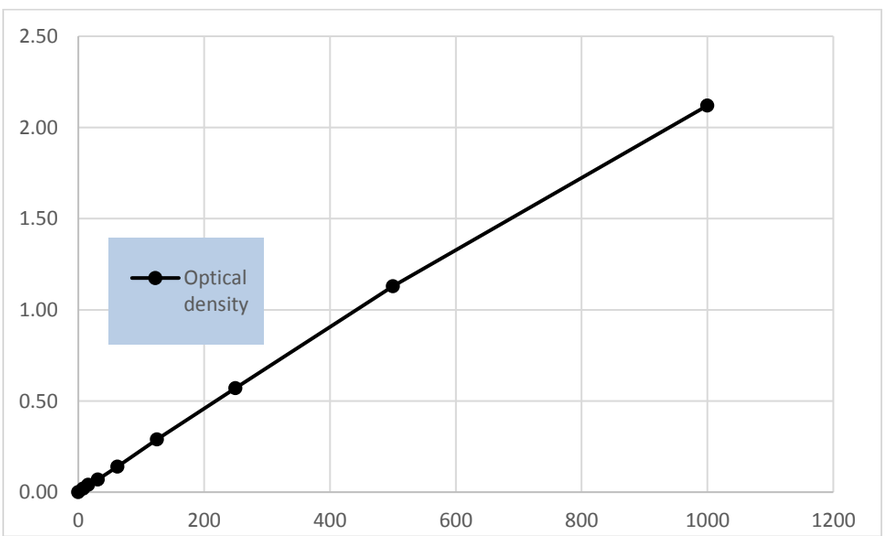
TYPICAL RESULTS

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

Sample	Mean OD	Net OD	IL-8 (pg/mL)
NSB	0.039	--	**--**
S0	0.039	0.00	**0**
S1	2.16	2.12	**1000**
S2	1.17	1.13	**500**
S3	0.60	0.57	**250**
S4	0.33	0.29	**125**
S5	0.18	0.14	**62.5**
S6	0.11	0.07	**31.3**
S7	0.07	0.04	**15.6**
S8	0.06	0.02	**7.8**

Typical Standard Curve

A typical standard curve is shown below. This curve must not be used to calculate IL-8 concentrations; each user must run a standard curve for each assay.



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by running serial dilutions of the analytes, including the cross-reactant, in the assay, fitting the resulting dose response curve(s) to a 4PL curve-fit and determining the ED_{50}. The ED_{50} of the cross-reactant was then divided by the determined ED_{50} of the IL-8 standard curve and multiplied by 100.

Analyte	% Cross Reactivity
IL-8	100
IL-2	2.4
IL-4	< 0.001
IL-6	0.86
IL-7	0.13
IL-10	0.01
GM-CSF	< 0.001
TNFα	< 0.001

Sensitivity

The sensitivity or limit of detection of the assay is 0.72pg/mL and was determined by interpolation at 2 standard deviations above the background (0pg/mL) of 20 zero standard replicates.

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

Intra-assay precision	
pg/mL	**%CV**
378.9	1.77
189.8	2.38
92.2	2.57

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

Inter-assay precision	
pg/mL	**%CV**
374.5	4.2
185.7	5.0
91.7	5.8

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