

Human IL-8 ELISA Kit

For the Quantitative Determination of Human Interleukin-8 (IL-8)
Concentrations in Serum, Plasma, Cell Culture Supernatant, and
Other Biological Fluids

Catalogue Number: EL10008

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE

This Human IL-8 ELISA kit is to be used for the *in vitro* quantitative determination of human interleukin-8 (IL-8) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin-8 (IL-8), also known as neutrophil attractant/activating protein (NAP-1), monocyte-derived neutrophil-activating peptide (MONAP), monocyte-derived neutrophil chemotactic factor (MDNCF), T lymphocyte chemotactic factor (TCF) and leukocyte adhesion inhibitor (LAI), is a member of the chemokine superfamily which selectively chemoattract and activate specific leukocyte subpopulations (1,2). All of these cytokines have four conserved cysteines and two distinguishable subfamilies. These two subfamilies are dependent on the position of the first two cysteines, which are either separated by one amino acid (C-X-C proteins) or are adjacent (CC-protein) to each other. The members of the two subfamilies differ in their target cell selectivity as well as the chromosomal location of their genes (chromosome 4 for the C-X-C proteins and chromosome 17 for the C-C proteins). IL-8 belongs to the C-X-C subfamily along with platelet factor 4 (PF4), platelet basic protein (PBP), connective-tissue-activating peptide III (CTAPIII), β -thromboglobulin, neutrophil-activating peptide-2 (NAP-2), ENA-78 (3), three closely related MGSA/CRO gene products (GRO- α , GRO- β , GRO- γ), and γ -interferon-inducible protein (γ -IP-10)(4). The members of the C-C chemokines are mainly chemotactic for monocytes whereas the C-X-C chemokines except for IP10 and PF4, chemoattract and activate neutrophils. In addition to the effect on neutrophils, IL-8 has been reported to be a less potent chemoattractant for T lymphocytes (5).

IL-8 is produced by many cells in response to inflammatory stimuli such as IL-1 β or TNF- α and to various types of mitogen, lectins, crystals, viruses, and phorbol esters (PMA). Many cell types that produce IL-8 in response to these stimuli can include: monocytes/macrophages, T lymphocytes, neutrophils, fibroblasts, keratinocytes, hepacytes, chondrocytes, endothelial cells, glioblastoma cells, and mesothelial cells (6).

The IL-8 predominant form secreted by stimulated monocytes has 72 residues (MW=8385), whereas the predominant form secreted by IL-1 stimulated endothelial cells has 77 residues (MW=8922). These variants have similar biological activities, although the 72-residue form of IL-8 appears to be 2 to 10 fold more potent than the 77-residue form depending on the type of assay used (7).

Various non-infectious human diseases are known to be associated with neutrophilia and/or neutrophil infiltration into organs. Examples of some of these human diseases include rheumatoid arthritis, gouty arthritis, psoriasis, glomerulonephritis, adult respiratory distress syndrome, immune vasculitis, inflammatory bowel disease, ischemia-reperfusion syndrome (including myocardial infarction and multiple organ failure), chorioretinitis, cystic fibrosis,

septic shock, acute meningococcal infections, alcoholic hepatitis and mediterranean fever (8). The presence of IL-8 has been positively identified in gouty arthritis, psoriatic scale, plasma from adult respiratory syndrome caused by sepsis, and serum from nephrotic syndrome as well as in the joint fluids from rheumatoid arthritis. The peripheral blood mononuclear cells (PBMC) obtained from patients undergoing an asthmatic attack have been shown to spontaneously produce *in vitro* IL-8-like molecules. The production of IL-8 triggers many other activities that contribute to these human diseases; however, IL-8 is not known to trigger systemic inflammatory reactions such as fever, acute phase protein induction. Development of an accurate immunoassay for the quantitative determination of human IL-8 levels in cell culture supernatant, serum, plasma and other biological fluids is expected to be effectively used for the further investigation of the relationship between IL-8 and various inflammatory diseases.

This IL-8 ELISA is a 2.5 hour solid-phase immunoassay readily applicable to measure IL-8 levels in serum, plasma, cell culture supernatant, and other biological fluids ranging from 0 to 1600 pg/mL. It has shown no cross-reactivity with human monocyte chemotactic activating factor (MCAF) or RANTES (Regulated on Activation, Normal T-cell Expressed, and Secreted). This IL-8 ELISA is expected to be effectively used for further investigations into the relationship between IL-8 and various diseases.

PRINCIPLES OF THE ASSAY

This IL-8 enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for IL-8. Standards or samples are then added to the appropriate microtiter plate wells and incubated. IL-8 if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound IL-8 and other components of the sample. In order to quantify the amount of IL-8 present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody specific for IL-8 is added to each well to "sandwich" the IL-8 immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-8 and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm \pm 2nm.

In order to measure the concentration of IL-8 in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of

Optical Density (O.D.) versus IL-8 concentration (pg/mL). The concentration of IL-8 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

		96 tests
1.	IL-8 MICROTITER PLATE (Part 30012) _____ Pre-coated with anti-human IL-8 monoclonal antibody.	96 wells
2.	IL-8 CONJUGATE (Part 30013) _____ Anti-human IL-8 polyclonal antibody conjugated to horseradish peroxidase with preservative.	15 mL
3.	IL-8 STANDARD (Part 30014) _____ Recombinant human IL-8 (6.4 ng/vial) in a buffered protein base with preservative, lyophilized.	2 vials
4.	CALIBRATOR DILUENT I (Part 30003) _____ Animal serum with preservative. <i>For serum/plasma testing.</i>	22 mL
5.	CALIBRATOR DILUENT II (Part EL 30004) _____ Cell culture medium with calf serum and preservative. <i>For cell culture supernatant/urine testing.</i>	22 mL
6.	WASH BUFFER (20X) (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	60 mL
7.	SUBSTRATE A (Part 30006) _____ Buffered solution with H ₂ O ₂ .	11 mL
8.	SUBSTRATE B (Part 30007) _____ Buffered solution with TMB.	11 mL
9.	STOP SOLUTION (Part 30008) _____ 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material!	14 mL

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μ L and 50-200 μ L for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm \pm 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
Solid Wastes: Autoclave for 60 minutes at 121°C.
Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

- a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
- b) **Serum:** Blood should be drawn using standard venipuncture techniques and serum should be separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C), and serum extracted.
- c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate. *This IL-8 ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.*
 - Avoid grossly hemolytic, lipidic or turbid samples.
 - Serum, plasma, cell culture supernatant, and urine samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
 - When performing the assay, slowly bring samples to room temperature.
 - It is recommended that all samples be assayed in duplicate.
 - DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

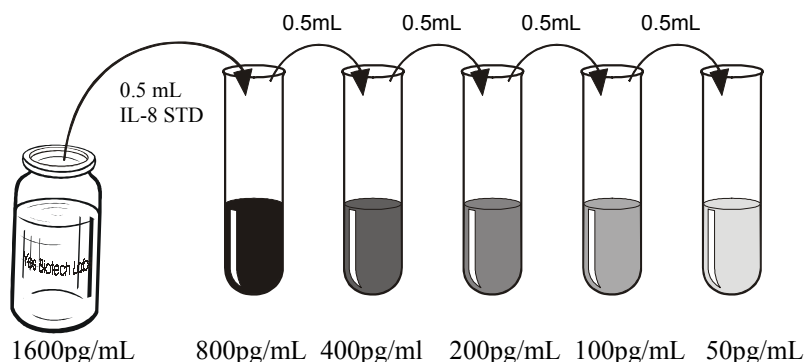
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the following table for the correct amount of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-8 Standard:**

- a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-8 Standard with either 4.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 1600 pg/mL. Allow the solution to sit for at least 15 minutes with gentle agitation prior to preparing the dilutions. Use within one hour of reconstituting. The IL-8 standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.
- b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (50 pg/mL to 1600 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-8 Standard will serve as the high standard (1600 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-8 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - 0 pg/mL (S1)	2A, 2B	Standard 5 - 400 pg/mL (S5)
1C, 1D	Standard 2 - 50 pg/mL (S2)	2C, 2D	Standard 6 - 800 pg/mL (S6)
1E, 1F	Standard 3 - 100 pg/mL (S3)	2E, 2F	Standard 7 - 1600 pg/mL (S7)
1G, 1H	Standard 4 - 200 pg/mL (S4)	2G-12H	IL-8 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

- Add 100 μ L of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.
- Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*
- Dispense two (2) drops or 100 μ L of conjugate to each well. Cover and incubate for 1 hour at room temperature.
- Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
- Repeat wash procedure as described in Step 3.

7. Add 100 μ L Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
8. Add 100 μ L Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-8 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-8 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of IL-8 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-8 concentration. If samples generate values greater than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

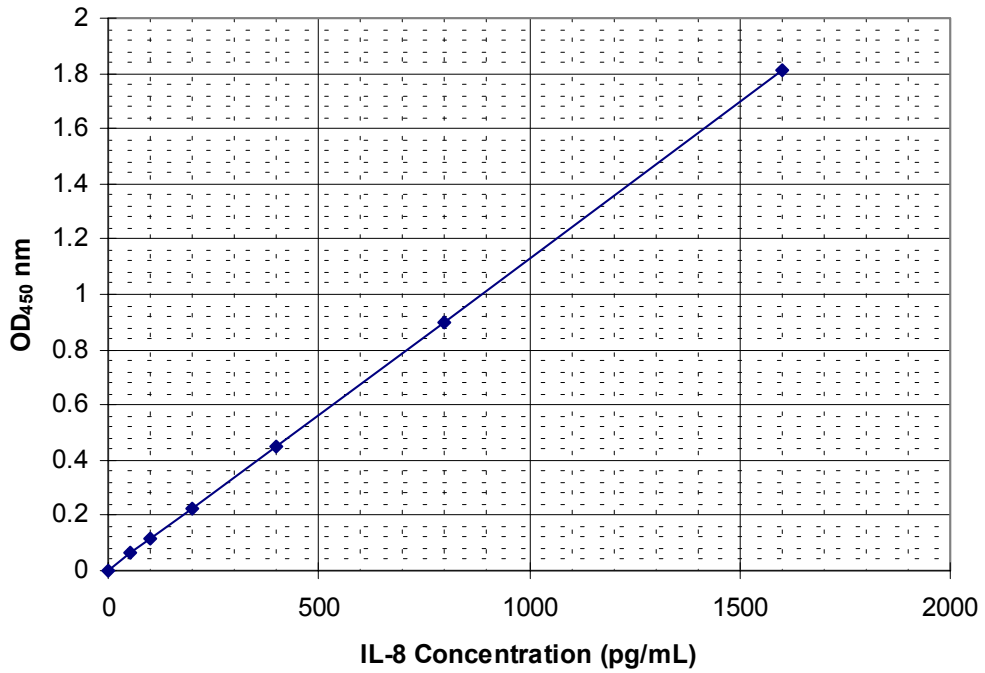
TYPICAL DATA

Results of a typical standard run of IL-8 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

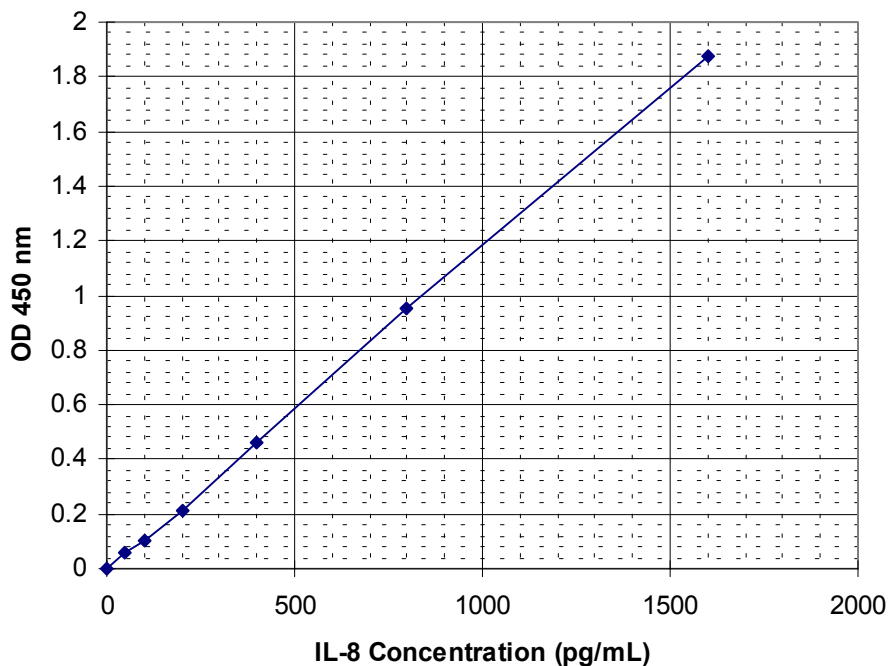
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.030, 0.038	0.034	0
50	0.102, 0.093	0.098	0.064
100	0.152, 0.148	0.150	0.116
200	0.249, 0.282	0.256	0.222
400	0.489, 0.482	0.485	0.451
800	0.950, 0.920	0.935	0.901
1600	1.848, 1.850	1.849	1.815



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.040, 0.036	0.038	0
50	0.096, 0.095	0.096	0.058
100	0.138, 0.138	0.138	0.100
200	0.244, 0.255	0.250	0.212
400	0.492, 0.504	0.498	0.460
800	0.986, 0.997	0.992	0.954
1600	1.892, 1.933	1.913	1.875



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 20 replicates in 1 assay.

Sample	Calibrator Diluent II assay		
	1	2	3
n	20	20	20
Mean (pg/mL)	103.10	312.40	993.20
Standard Deviation (pg/mL)	5.08	12.39	28.50
Coefficient of Variation (%)	4.90	4.00	2.90

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 20 different assays.

Sample	Calibrator Diluent II assay		
	1	2	3
n	20	20	20
Mean (pg/mL)	99.8	293.5	957.8
Standard Deviation (pg/mL)	9.1	18.3	61.8
Coefficient of Variation (%)	9.1	6.2	6.5

3. **RECOVERY**

The recovery of IL-8 within three different levels in various matrices was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture supernatant	105	85 - 123
Plasma	98	82 - 99
Serum	94	79 - 100

4. **SENSITIVITY**

The minimum detectable dose of IL-8 was determined by adding two standard deviations to the mean optical density value of the 20 zero-standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is *10 pg/mL* and using Calibrator Diluent II is *4 pg/mL*.

5. **SPECIFICITY**

This sandwich ELISA recognizes both natural and recombinant human IL-8. This kit exhibits no detectable cross-reactivity with human IL-1 β , EGF, MCAF, RANTES, and SAA.

6. **CALIBRATION**

This immunoassay is calibrated against a highly purified E. Coli expressed as a 72 amino-acid form (monocyte secreted) of recombinant human IL-8.

7. **EXPECTED NORMAL VALUES**

Biological samples from apparently healthy, normal individuals were collected and the average IL-8 concentration measured. Serum/plasma samples (n=29) are less than 20 pg/mL. One sample is 50 pg/mL.

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