



IL8 (Human) ELISA Kit

Catalog Number KA0115

96 assays

Version: 05

Intended for research use only

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Table of Contents

Introduction	3
Intended Use	3
Background	3
Principle of the Assay	5
General Information	7
Materials Supplied	7
Storage Instruction	7
Materials Required but Not Supplied	7
Precautions for Use	8
Assay Protocol	10
Reagent Preparation	10
Sample Preparation	12
Assay Procedure	13
Data Analysis	15
Calculation of Results	15
Performance Characteristics	18
Resources	22
Plate Layout	22

Introduction

Intended Use

The IL8 (Human) ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-8/NAP-1. The IL8 (Human) ELISA Kit is for research use only. Not for diagnostic or therapeutic procedures.

Background

Interleukin-8/Neutrophil-Activating Peptide-1 selectively stimulates the ability of neutrophils and T-lymphocytes to invade injured or inflamed tissue. IL-8 purified from peripheral blood monocytes is a 10 kDa protein showing at least 4 variant forms (77, 72, 70, and 69 amino acids, respectively) which differ in the length of the N-terminal and readily form dimers in solution. Two cystin-bridges are essential for the biological activity of IL-8. Exogenous stimuli like LPS, but also IL-1, TNF α and TNF β induce the secretion of IL-8 in a variety of different cell types including monocytes, endothelial and epithelial cells, peripheral blood mononuclear cells, dermal fibroblasts, keratinocytes, neutrophils, hepatocytes, synovial cells, and T-lymphocytes. When IL-8 was subcutaneously injected into rats, both lymphocytes and neutrophils migrated to the site of injection within 3 hours. At lower dosages, only lymphocytes migrated towards the site of injection, while at higher dosages primarily neutrophils were attracted. It was found that T-lymphocytes are 10 times more sensitive to IL-8 than neutrophils. IL-8 exerts its effects via specific cell membrane receptors (chemotactic agonist receptor-family) with homogeneous high-affinity activity and two binding sites for its ligand. The receptor density is determined by the cell type and ranges from 300 on T-lymphocytes up to 20.000 on neutrophils. After binding of IL-8, the receptor expression is downregulated >90 % within 10 minutes at 37°C, together with the internalization of the ligand. IL-8 is proteolytically degraded in the cytoplasm and released into the culture medium as soluble fragments. The IL-8 receptors are probably recycled. Besides its chemotactic influence, IL-8 exerts other distinct characteristics. In neutrophils it triggers the secretion of superoxide anions and lysosomal enzymes, thereby indirectly augmenting the permeability of blood vessels, and IL-8 enhances the fungicidal activity against *Candida albicans*. Neutrophils are more readily liberated from the bone marrow reservoir under the influence of this cytokine. In vitro, IL-8 stimulates a rapid Mac-1 as well as CR1, p150,95 and LFA-1 expression on neutrophils which enables the adherence to activated vascular endothelial cells expressing e.g. ICAM-1. This may account for the accumulation of neutrophils at IL-8 injection sites. Other findings suggest that endothelial-derived IL-8 may function to attenuate inflammatory events at the interface between vessel wall and blood, via inhibiting neutrophil adhesion to cytokine-activated endothelial monolayers. Therefore these cells seem to be protected against neutrophil-mediated damage. In basophils, besides its chemotactic effects, IL-8 stimulates the histamin liberation in atopic as well as healthy persons.

The property of IL-8 to stimulate movement of neutrophils across endothelial monolayers in vitro supports the concept of a central role for this molecule in the accumulation of neutrophils at inflammatory lesions in vivo. Data indicate that IL-8 may participate in the pathogenesis of rheumatoid arthritis via the induction of neutrophil-mediated cartilage damage, and psoriasis. A causative involvement of IL-8 is found within a broad

range of clinico-pathological conditions: adult respiratory distress syndrome, asthma, bacterial infections, bladder cancer, blood incompatibility, contact dermatitis, empyema, graft rejection, glomerulonephritis, gout, hemolytic uremic syndrome, immune vasculitis, inflammatory bowel disease, influenza virus infection, Jarisch-Herxheimer reaction, malignancies of the central nervous system, myocardial infarction, placental infection, sepsis, uveitis, among others.

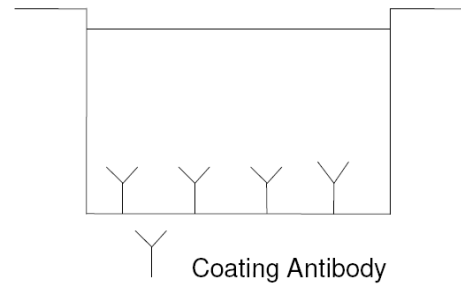
Due to the nowadays known biological properties of IL-8, this cytokine, especially in combinations with other neutrophil activating agents, may prove helpful in the treatment of patients suffering from granulocytopenia, severe infections against which antibiotics are not effective, and immunodeficiency caused by HI-virus.

Principle of the Assay

An anti-human IL-8/NAP-1 coating antibody is adsorbed onto microwells.

Figure 1

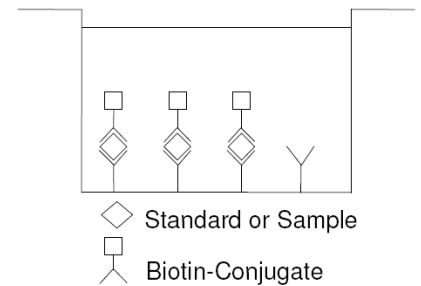
Coated Microwell



Human IL-8/NAP-1 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-8/NAP-1 antibody is added and binds to human IL-8/NAP-1 captured by the first antibody.

Figure 2

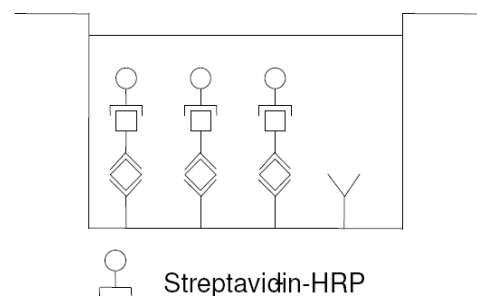
First Incubation



Following incubation unbound biotin-conjugated anti-human IL-8/NAP-1 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-8/NAP-1 antibody.

Figure 3

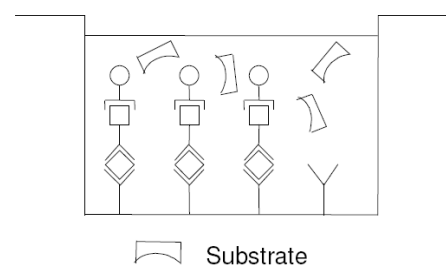
Second Incubation



Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

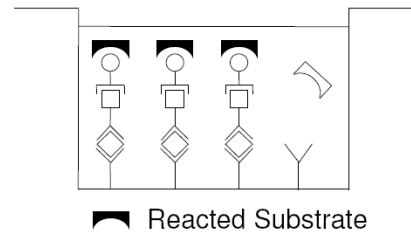
Figure 4

Third Incubation



A coloured product is formed in proportion to the amount of human IL-8/NAP-1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-8/NAP-1 standard dilutions and human IL-8/NAP-1 sample concentration determined.

Figure 5



General Information

Materials Supplied

List of component

Component	Amount
Microwell Plate coated with monoclonal antibody to human IL-8/NAP-1	1 aluminium pouch
Biotin-Conjugate anti-human IL-8/NAP-1 polyclonal antibody	70 µL
Streptavidin-HRP	150 µL
human IL-8/NAP-1 Standard, 100 ng/mL	100 µL x 2
Control, high, lyophilized	1 vial
Control, low, lyophilized	1 vial
Sample Diluent	12 mL
Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)	5 mL
Wash Buffer Concentrate 20x (PBS with 1% Tween 20)	50 mL
Substrate Solution (tetramethyl-benzidine)	15 mL
Stop Solution (1M Phosphoric acid)	15 mL
Adhesive Films	4 slices

Note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard and Biotin-Conjugate vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

Storage Instruction

Store kit reagents between 2° and 8°C except controls. Store lyophilized control at -20°C.

Immediately after use remaining reagents should be returned to cold storage (2° to 8°C), controls to -20°C, respectively. Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Materials Required but Not Supplied

- ✓ 5 mL and 10 mL graduated pipettes
- ✓ 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- ✓ 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- ✓ Multichannel micropipette reservoir
- ✓ Beakers, flasks, cylinders necessary for preparation of reagents
- ✓ Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- ✓ Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- ✓ Glass-distilled or deionized water
- ✓ Statistical calculator with program to perform regression analysis

Precautions for Use

- Precautions
- ✓ All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- ✓ Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- ✓ Do not mix or substitute reagents with those from other lots or other sources.
- ✓ Do not use kit reagents beyond expiration date on label.
- ✓ Do not expose kit reagents to strong light during storage or incubation.
- ✓ Do not pipette by mouth.
- ✓ Do not eat or smoke in areas where kit reagents or samples are handled.
- ✓ Avoid contact of skin or mucous membranes with kit reagents or specimens.
- ✓ Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- ✓ Avoid contact of substrate solution with oxidizing agents and metal.
- ✓ Avoid splashing or generation of aerosols.
- ✓ In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- ✓ Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- ✓ Exposure to acid inactivates the conjugate.
- ✓ Glass-distilled water or deionized water must be used for reagent preparation.
- ✓ Substrate solution must be at room temperature prior to use.
- ✓ Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- ✓ Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

- Limitations
- ✓ Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- ✓ Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- ✓ Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- ✓ Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- ✓ The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Assay Protocol

Reagent Preparation

Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

- Wash Buffer (1x)

Pour entire contents (50 mL) of the Wash Buffer Concentrate (1x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1-6	25	475
1-12	50	950

- Assay Buffer (1x)

Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1-6	2.5	47.5
1-12	5.0	95.0

- Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1-6	0.03	2.97
1-12	0.06	5.94

Please note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard and Biotin-Conjugate vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

- Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1-6	0.03	5.97
1-12	0.06	11.94

- Human IL-8/NAP-1 Standard

The concentrated human IL-8/NAP-1 standard must be diluted 1:50 with Assay Buffer (1x) just prior to use in a clean plastic test tube according to the following dilution scheme:

20 μ L concentrated human IL-8/NAP-1 standard + 980 μ L Assay Buffer (1x). Shake gently to mix (concentration of standard = 2000 pg/mL).

Please note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the standard vials. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

Standard dilutions can be prepared directly on the microwell plate or alternatively in tubes.

External Standard Dilution

Label 7 tubes, one for each standard point.

S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 μ L of Sample Diluent into each tube.

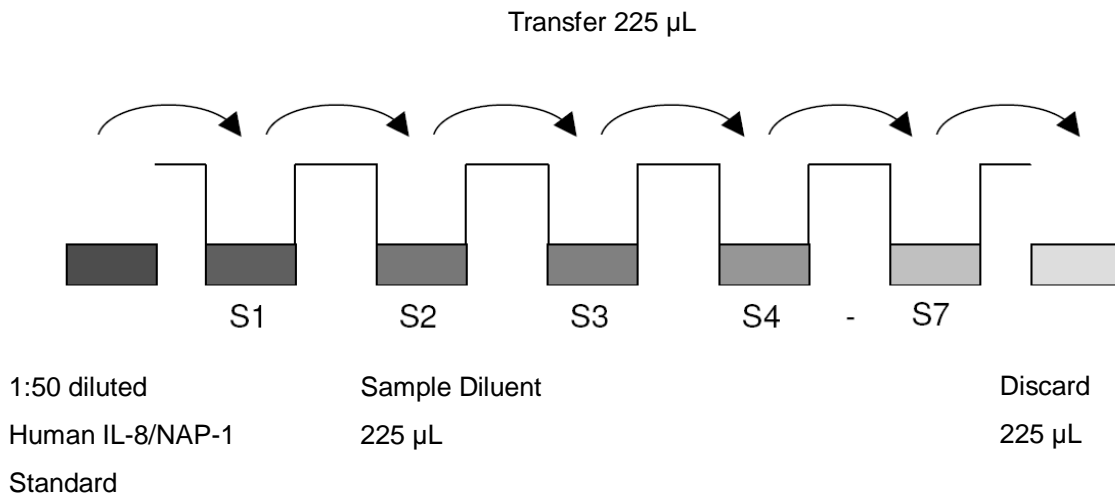
Pipette 225 μ L of diluted standard (concentration = 2000 pg/mL) into the first tube, labelled S1, and mix (concentration of standard 1 = 1000 pg/mL).

Pipette 225 μ L of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.

Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

Sample Diluent serves as blank.

Figure 6



- Controls

Reconstitute by adding 350 μ L distilled water to lyophilized controls (10-30 minutes). Swirl or mix gently to ensure complete and homogeneous solubilization. Further treat the controls like your samples in the assay. For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at -20°C . Avoid repeated freeze and thaw cycles.

Sample Preparation

Cell culture supernatant *, serum, plasma (EDTA and heparin) and amniotic fluid were tested with this assay. Other biological samples might be suitable for use in the assay.

Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IL-8/NAP-1.

If samples are to be run within 24 hours, they may be stored at 2° to 8°C . Avoid repeated freeze-thaw cycles.

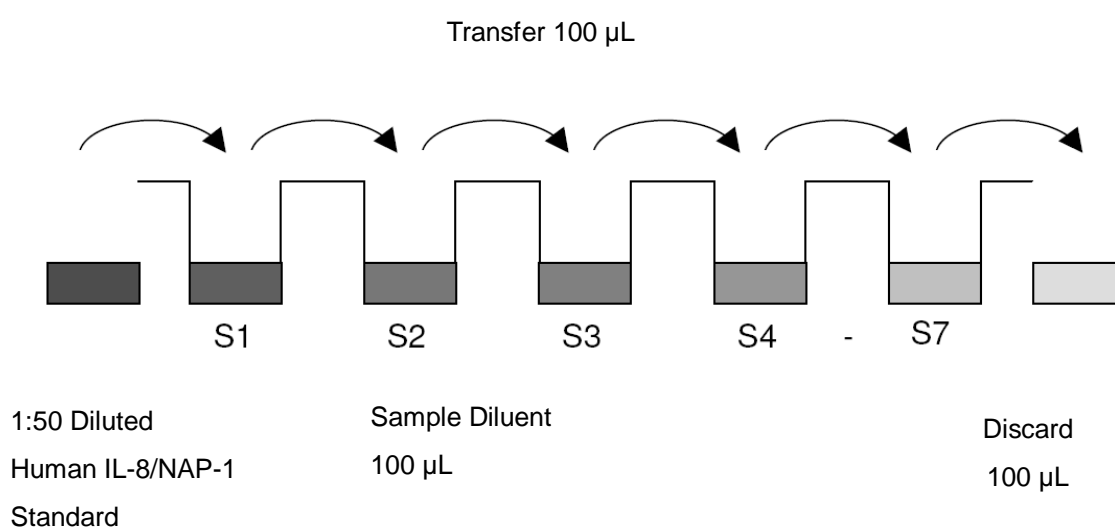
Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

* Elevated blank signal was observed when using DMEM cell culture medium. No elevated blank signal was observed when using RPMI cell culture medium.

Assay Procedure

- Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
- Wash the microwell strips twice with approximately 400 μL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
- Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes): Add 100 μL of Sample Diluent in duplicate to all standard wells. Pipette 100 μL of prepared standard (see Preparation of Standard, concentration = 2000 pg/mL) in duplicate into well A1 and A2 (see Plate layout). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1000.0 pg/mL), and transfer 100 μL to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IL-8/NAP-1 standard dilutions ranging from 1000.0 to 15.6 pg/mL . Discard 100 μL of the contents from the last microwells (G1, G2) used.

Figure 7



In case of an external standard dilution, pipette 100 μL of these standard dilutions (S1 - S7) in the standard wells according to Plate layout.

4. Add 100 μL of Sample Diluent in duplicate to the blank wells.
5. Add 50 μL of Sample Diluent to the sample wells.
6. Add 50 μL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 μL of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.
10. Prepare Streptavidin-HRP.
11. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 2. of the test protocol. Proceed immediately to the next step.
12. Add 100 μL of diluted Streptavidin-HRP to all wells, including the blank wells.
13. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 400 rpm.
14. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 2. of the test protocol. Proceed immediately to the next step.
15. Pipette 100 μL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

17. Stop the enzyme reaction by quickly pipetting 100 μL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
18. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Data Analysis

Calculation of Results

- ✓ Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- ✓ Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-8/NAP-1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- ✓ To determine the concentration of circulating human IL-8/NAP-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-8/NAP-1 concentration.
- ✓ If instructions in this protocol have been followed samples have been diluted 1:2 (50 μ L sample + 50 μ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- ✓ Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IL-8/NAP-1 levels. Such samples require further external predilution according to expected human IL-8/NAP-1 values with Sample Diluent in order to precisely quantitate the actual human IL-8/NAP-1 level.
- ✓ It is suggested that each testing facility establishes a control sample of known human IL-8/NAP-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- ✓ A representative standard curve is shown in Figure 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 8

Representative standard curve for IL8 (Human) ELISA Kit. Human IL-8/NAP-1 was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

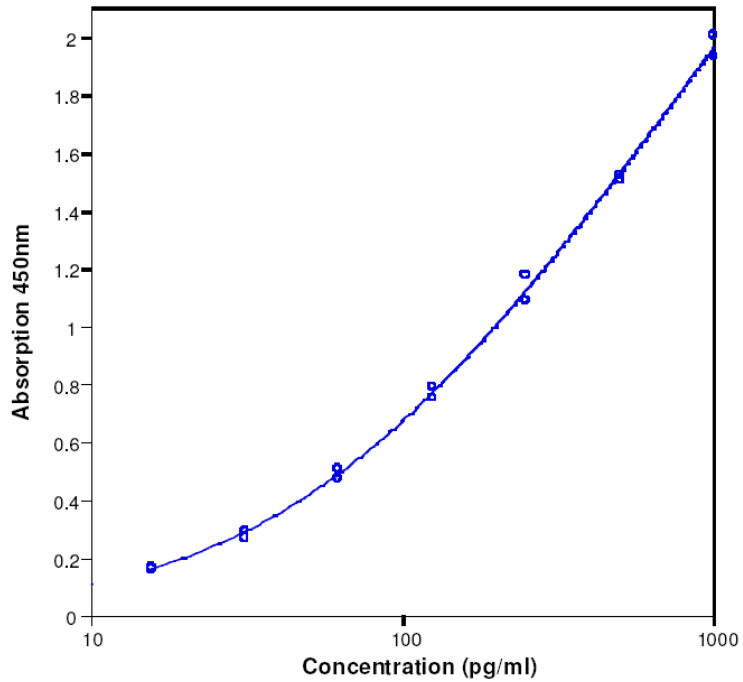


Table 1

Typical data using the IL8 (Human) ELISA Kit

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human IL-8/NAP-1 Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1000.0	2.003 1.931	1.967	1.8
2	500.0	1.522 1.504	1.513	0.6
3	250.0	1.175 1.088	1.132	3.8
4	125.0	0.787 0.751	0.769	2.3
5	62.5	0.507 0.472	0.490	3.6
6	31.3	0.292 0.266	0.279	4.7
7	15.6	0.167 0.163	0.165	1.2
Blank	0	0.038 0.042	0.040	5.0

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

Performance Characteristics

- Sensitivity

The limit of detection of human IL-8/NAP-1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 2.0 pg/mL (mean of 6 independent assays).

- Precision

- ✓ Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human IL-8/NAP-1. 2 standard curves were run on each plate. Data below show the mean human IL-8/NAP-1 concentration and the coefficient of variation for each sample (see Table 2). The calculated overall intra-assay coefficient of variation was 6.3%.

Table 2

The mean human IL-8/NAP-1 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human IL-8/NAP-1 Concentration (pg/mL)	Coefficient of Variation (%)
1	1	742.8	7
	2	712.0	7
	3	615.3	8
2	1	348.7	4
	2	353.1	8
	3	384.3	3
3	1	184.5	5
	2	183.9	5
	3	187.7	6
4	1	80.0	7
	2	65.8	8
	3	74.6	8
5	1	44.3	4
	2	34.6	10
	3	41.7	7

6	1	520.4	4
	2	477.9	6
	3	615.1	5
7	1	273.0	4
	2	251.2	3
	3	291.8	11
8	1	316.0	9
	2	313.3	6
	3	260.0	9

✓ Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human IL-8/NAP-1. 2 standard curves were run on each plate. Data below show the mean human IL-8/NAP-1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 3). The calculated overall inter-assay coefficient of variation was 8.7%.

Table 3

The mean human IL-8/NAP-1 concentration and the coefficient of variation of each sample

Sample	Mean Human IL-8/NAP-1 Concentration (pg/mL)	Coefficient of Variation (%)
1	690.0	9.6
2	362.0	5.4
3	185.4	1.1
4	73.5	9.7
5	40.2	12.5
6	537.8	13.1
7	272.0	7.5
8	296.4	10.7

- Spiking Recovery

The spike recovery was evaluated by spiking 4 levels of human IL-8/NAP-1 into serum samples. Recoveries were determined in 3 independent experiments with 6 replicates each. The amount of endogenous human IL-8/NAP-1 in unspiked serum was subtracted from the spike values. The recovery ranged from 72% to 125% with an overall mean recovery of 88%.

- Dilution Linearity

4 serum samples with different levels of human IL-8/NAP-1 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged from 90% to 119% with an overall recovery of 107% (see Table 4).

Table 4

Sample	Dilution	Expected Human IL-8/NAP-1 Concentration (pg/mL)	Observed Human IL-8/NAP-1 Concentration (pg/mL)	Recovery of Expected Human IL-8/NAP-1 Concentration (%)
1	1:2	---	771.7	--
	1:4	385.9	402.4	104
	1:8	201.2	217.8	108
	1:16	108.9	104.3	96
2	1:2	---	369.9	--
	1:4	185.0	194.0	105
	1:8	97.0	102.6	106
	1:16	51.3	61.1	119
3	1:2	---	49.7	--
	1:4	24.8	22.5	90
	1:8	11.2	11.6	104
	1:16	5.8	6.6	114
4	1:2	---	315.9	--
	1:4	158.0	175.6	111
	1:8	87.8	99.5	113
	1:16	49.8	59.0	119

- Sample Stability

- ✓ Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human IL-8/NAP-1 levels determined. There was no significant loss of human IL-8/NAP-1 immunoreactivity detected by freezing and thawing.

✓ Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-8/NAP-1 level determined after 24 h. There was no significant loss of human IL-8/NAP-1 immunoreactivity detected during storage under above conditions.

- Specificity

The assay detects both natural and recombinant human IL-8/NAP-1. The interference of circulating factors of the immune system with high homology to IL-8/NAP-1 was evaluated by spiking these proteins at physiologically relevant concentrations (up to 1000 ng/mL) into a human IL-8/NAP-1 positive serum.

There was no crossreactivity detected with connective tissue activating peptide 3, platelet factor 4 and neutrophil activating peptide 2 as well as IL-6, IL-2R, rhTNF-β and CD8.

- Expected Values

Panels of 40 serum as well as EDTA and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for human IL-8/NAP-1.

Elevated human IL-8/NAP-1 levels depend on the type of immunological disorder. The levels measured may vary with the sample collection used. For detected human IL-8/NAP-1 levels see Table 5.

Table 5

Sample Matrix	Number of Samples Evaluated	Range (pg/mL)	% Detectable	Mean of Detectable (pg/mL)
Serum	40	34.8 - 666.4	22.5	114.0
Plasma (EDTA)	40	nd *- 97.4	2.5	--
Plasma (Heparin)	40	nd *- 34.8	2.5	--

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

Resources

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1 (1000.0 pg/mL)	Standard 1 (1000.0 pg/mL)	Sample 1	Sample 1	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	Standard 2 (500.0 pg/mL)	Standard 2 (500.0 pg/mL)	Sample 2	Sample 2	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	Standard 3 (250.0 pg/mL)	Standard 3 (250.0 pg/mL)	Sample 3	Sample 3	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	Standard 4 (125.0 pg/mL)	Standard 4 (125.0 pg/mL)	Sample 4	Sample 4	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	Standard 5 (62.5 pg/mL)	Standard 5 (62.5 pg/mL)	Sample 5	Sample 5	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	Standard 6 (31.3 pg/mL)	Standard 6 (31.3 pg/mL)	Sample 6	Sample 6	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	Standard 7 (15.6 pg/mL)	Standard 7 (15.6 pg/mL)	Sample 7	Sample 7	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	Blank	Blank	Sample 8	Sample 8	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample