



## **ELISA PRODUCT INFORMATION & MANUAL**

### **Human CXCL8/IL-8 ELISA Kit (Colorimetric) *NBP1-83743***

Enzyme-linked Immunosorbent Assay for quantitative  
detection. For research use only.

Not for diagnostic or therapeutic procedures.

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# Human CXCL8/IL-8 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human CXCL8/IL-8

Catalog Number NBP1-83743

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from Technical Support.

## Product description

The Human CXCL8/IL-8 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-8.

## Summary

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 10kDa protein showing at least four 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 alpha and TNF beta, 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 down-regulated >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., sICAM-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.

## Principles of the test

An anti-human CXCL8/IL-8 coating antibody is adsorbed onto microwells.

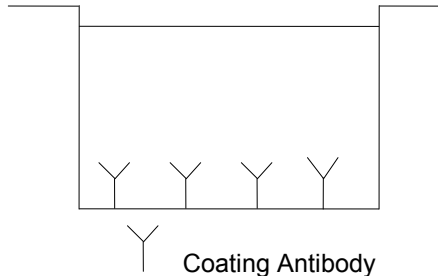


Fig. 1 Coated microwell.

Human CXCL8/IL-8 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human CXCL8/IL-8 antibody is added and binds to human CXCL8/IL-8 captured by the first antibody.

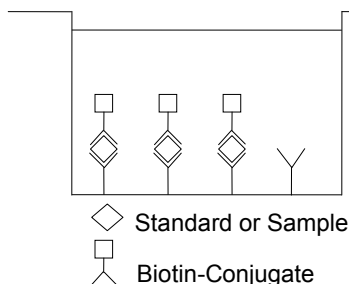


Fig. 2 First incubation.

Following incubation unbound biotin-conjugated anti-human CXCL8/IL-8 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human CXCL8/IL-8 antibody.

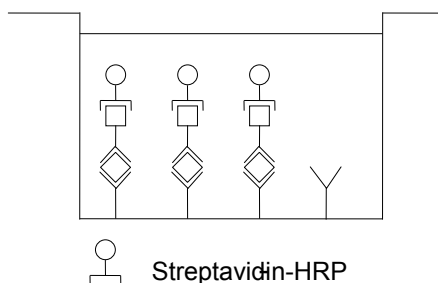


Fig. 3 Second incubation.

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

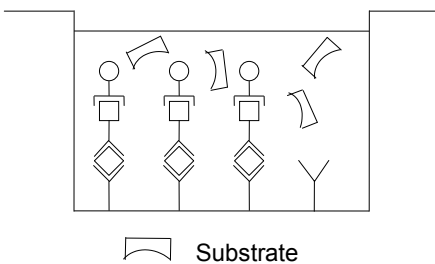


Fig. 4 Third incubation.

A colored product is formed in proportion to the amount of human CXCL8/CXCL8/IL-8 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 CXCL8/IL-8 standard dilutions and human CXCL8/IL-8 sample concentration determined.

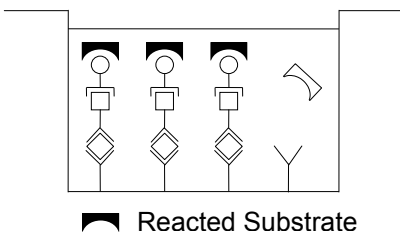


Fig. 5 Stop reaction.

## Reagents provided

### Reagents for human CXCL8/IL-8 ELISA NBP1-83743

#### (96 tests)

- 1 aluminum pouch with a Microwell Plate (12 strips with 8 well each) coated with monoclonal antibody to human CXCL8/IL-8
- 1 vial (70  $\mu$ L) Biotin-Conjugate anti-human CXCL8/IL-8 polyclonal antibody
- 1 vial (150  $\mu$ L) Streptavidin-HRP
- 2 vials (100  $\mu$ L) human CXCL8/IL-8 Standard, 100 ng/mL
- 1 vial Control high, lyophilized
- 1 vial Control low, lyophilized
- 1 vial (12 mL) Sample Diluent
- 1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)
- 1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)
- 1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)
- 1 vial (15 mL) Stop Solution (1M Phosphoric acid)
- 4 Adhesive Films

**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.

### Reagents for human CXCL8/IL-8 ELISA NBP1-83743

#### (10x96 tests)

- 10 aluminum pouches with a Microwell Plate (12 strips with 8 well each) coated with monoclonal antibody to human CXCL8/IL-8
- 10 vials (70  $\mu$ L) Biotin-Conjugate anti-human CXCL8/IL-8 polyclonal antibody
- 10 vials (150  $\mu$ L) Streptavidin-HRP
- 10 vials (100  $\mu$ L) human CXCL8/IL-8 Standard, 100 ng/mL
- 10 vials Control high, lyophilized
- 10 vials Control low, lyophilized
- 10 vials (12 mL) Sample Diluent
- 3 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)
- 4 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

20 Adhesive Films

**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 instructions – ELISA kit

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

Immediately after use remaining reagents should be returned to cold storage (2–8°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.

## Sample collection and storage instructions

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.

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

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 samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human CXCL8/IL-8. If samples are to be run within 24 hours, they may be stored at 2–8°C (refer to “Sample stability” on page 6). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

## Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
- 50  $\mu$ L to 300  $\mu$ L adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and 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

- 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 pipet 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that 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 samples and all potentially contaminated materials as if 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.

## Preparation of reagents

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

## Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (1x) into a clean 1,000 mL graduated cylinder. Bring to final volume of 1,000 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2–25°C. The Wash Buffer (1x) is stable for 30 days.
4. 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)

1. 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.
2. Store at 2–8°C. The Assay Buffer (1x) is stable for 30 days.
3. 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

**Note:** The Biotin-Conjugate should be used within 30 minutes after dilution.

**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.

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

## Streptavidin-HRP

**Note:** 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 CXCL8/IL-8 standard

1. The concentrated human CXCL8/IL-8 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 µL concentrated human CXCL8/IL-8 standard + 980 µL Assay Buffer (1x)

Shake gently to mix (concentration of standard = 2,000 pg/mL).

**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.

2. Standard dilutions can be prepared directly on the microwell plate (see “Test protocol” on page 4) or alternatively in tubes (see “External standard dilution” on page 3).

## External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of diluted standard (concentration = 2000 pg/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 1000 pg/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6).

Sample Diluent serves as blank

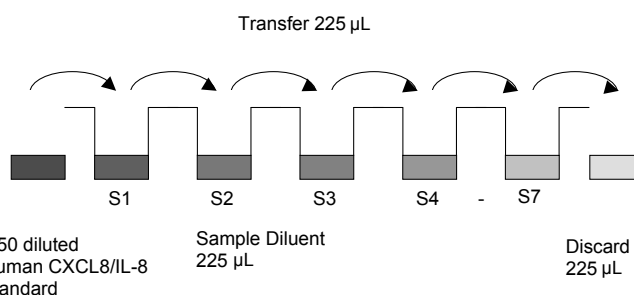


Fig. 6 Dilute standards - tubes.

## Controls

Reconstitute by adding 350  $\mu\text{L}$  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 refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at  $-20^{\circ}\text{C}$ . Avoid repeated freeze and thaw cycles.

## Test protocol

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

- 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  $2-8^{\circ}\text{C}$  sealed tightly.
- Wash the microwell strips twice with approximately 400  $\mu\text{L}$  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, see “External standard dilution” on page 3):  
Add 100  $\mu\text{L}$  of Sample Diluent in duplicate to all standard wells. Pipette 100  $\mu\text{L}$  of prepared standard (see “Human CXCL8/IL-8 standard” on page 3, concentration = 2,000.0 pg/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1,000.0 pg/mL), and transfer 100  $\mu\text{L}$  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 CXCL8/IL-8 standard dilutions ranging from 1,000.0 to 15.6 pg/mL. Discard 100  $\mu\text{L}$  of the contents from the last microwells (G1, G2) used.

Transfer 100  $\mu\text{L}$

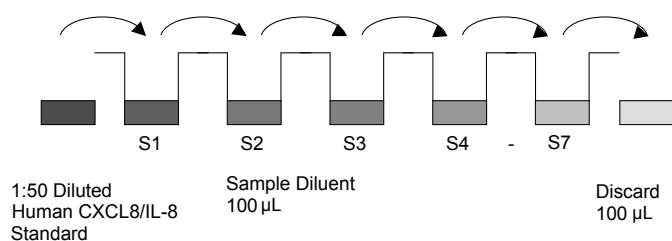


Fig. 7 Dilute standards - microwell plate.

In case of an external standard dilution (see “External standard dilution” on page 3), pipette 100  $\mu\text{L}$  of these standard dilutions (S1–S7) in the standard wells according to Table 1.

**Table 1** Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 1,000.0 pg/mL	Standard 1 1,000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 500.0 pg/mL	Standard 2 500.0 pg/mL	Sample 2	Sample 2
C	Standard 3 250.0 pg/mL	Standard 3 250.0 pg/mL	Sample 3	Sample 3
D	Standard 4 125.0 pg/mL	Standard 4 125.0 pg/mL	Sample 4	Sample 4
E	Standard 5 62.5 pg/mL	Standard 5 62.5 pg/mL	Sample 5	Sample 5
F	Standard 6 31.3 pg/mL	Standard 6 31.3 pg/mL	Sample 6	Sample 6
G	Standard 7 15.6 pg/mL	Standard 7 15.6 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- Add 100  $\mu\text{L}$  of Sample Diluent in duplicate to the blank wells.
- Add 50  $\mu\text{L}$  of Sample Diluent to the sample wells.
- Add 50  $\mu\text{L}$  of each sample in duplicate to the sample wells.
- Prepare Biotin-Conjugate (see “Biotin-Conjugate” on page 3).
- Add 50  $\mu\text{L}$  of Biotin-Conjugate to all wells.
- Cover with an adhesive film and incubate at room temperature ( $18-25^{\circ}\text{C}$ ) for 2 hours, if available on a microplate shaker.
- Prepare Streptavidin-HRP (see “Streptavidin-HRP” on page 3).
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 2. Proceed immediately to the next step.
- Add 100  $\mu\text{L}$  of diluted Streptavidin-HRP to all wells, including the blank wells.
- Cover with an adhesive film and incubate at room temperature ( $18-25^{\circ}\text{C}$ ) for 1 hour, if available on a microplate shaker.
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 2. Proceed immediately to the next step.
- Pipette 100  $\mu\text{L}$  of TMB Substrate Solution to all wells.
- Incubate the microwell strips at room temperature ( $18-25^{\circ}\text{C}$ ) for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color 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 color. Alternatively the color 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.

- Stop the enzyme reaction by quickly pipetting 100  $\mu\text{L}$  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  $2-8^{\circ}\text{C}$  in the dark.
- 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.

## Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human CXCL8/IL-8 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve is recommended).
- To determine the concentration of circulating human CXCL8/IL-8 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 CXCL8/IL-8 concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:2 (50  $\mu$ L sample + 50  $\mu$ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor ( $\times 2$ ).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human CXCL8/IL-8 levels. Such samples require further external predilution according to expected human CXCL8/IL-8 values with Sample Diluent in order to precisely quantitate the actual human CXCL8/IL-8 level.
- It is suggested that each testing facility establishes a control sample of known human CXCL8/IL-8 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.

**Note:** Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

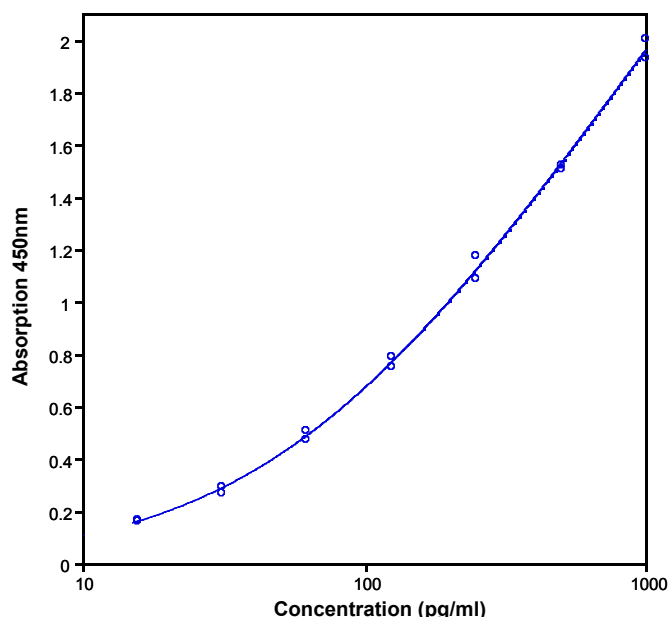


Fig. 8 Representative standard curve for Human CXCL8/IL-8 ELISA Kit. Human CXCL8/IL-8 was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the Human CXCL8/IL-8 ELISA Kit (measuring wavelength of 450 nm, reference wavelength of 620 nm).

Standard	Human CXCL8/IL-8 Conc. (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1,000.0	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 color intensity. Values measured are still valid.

## Limitations

- Because 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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

## Performance characteristics

### Sensitivity

The limit of detection of human CXCL8/IL-8 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).

### Reproducibility

#### 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 CXCL8/IL-8. Two standard curves were run on each plate. Data below show the mean human CXCL8/IL-8 concentration and the coefficient of variation for each

sample. The calculated overall intra-assay coefficient of variation was 6.3%.

**Table 3** The mean human CXCL8/IL-8 concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean 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 CXCL8/IL-8. Two standard curves were run on each plate. Data below show the mean human CXCL8/IL-8 concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 8.7%.

**Table 4** The mean human CXCL8/IL-8 concentration and the coefficient of variation of each sample.

Sample	Mean 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

#### Spike recovery

The spike recovery was evaluated by spiking 4 levels of human CXCL8/IL-8 into serum samples. Recoveries were determined in 3 independent experiments with 6 replicates each. The amount of endogenous human CXCL8/IL-8 in unspiked serum was subtracted from the spike values. The recovery ranged from 72–125% with an overall mean recovery of 88%.

#### Dilution parallelism

Four serum samples with different levels of human CXCL8/IL-8 were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 90% to 119% with an overall recovery of 107%.

Sample	Dilution	Expected human IL-8 concentration (pg/mL)	Observed human IL-8 concentration (pg/mL)	Recovery of expected human IL-8 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 CXCL8/IL-8 levels determined. There was no significant loss of human CXCL8/IL-8 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, and at 37°C, and the human CXCL8/IL-8 level determined after 24 hours. There was no significant loss of human CXCL8/IL-8 immunoreactivity detected during storage under above conditions.

#### Specificity

The assay detects both natural and recombinant human CXCL8/IL-8. The interference of circulating factors of the immune system with high homology to CXCL8/IL-8 was evaluated by spiking these proteins at physiologically relevant concentrations (up to 1000 pg/mL) into a human CXCL8/IL-8 positive serum. No cross-reactivity was detected with connective tissue activating peptide 3, platelet factor 4, and neutrophil activating peptide 2, as well as IL-6, IL-2R, rhTNF beta, 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 CXCL8/IL-8. Elevated human CXCL8/IL-8 levels depend on the type of immunological disorder. The levels measured may vary with the sample collection used.

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 <sup>[1]</sup> –97.4	2.5	–
Plasma (heparin)	40	nd <sup>[1]</sup> –34.8	2.5	–

<sup>[1]</sup> nd = nondetectable, samples measured below the lowest standard point are considered to be nondetectable

## Reagent preparation summary

### Wash buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

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

### Assay buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

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

### Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

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

### Streptavidin-HRP

Make a 1:200 dilution of Streptavidin-HRP in Assay Buffer (1x):

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

### Human CXCL8/IL-8 standard

The concentrated human CXCL8/IL-8 standard must be diluted 1:50 with Assay Buffer (1x).

### Controls

Add 350  $\mu$ L distilled water to lyophilized controls.

## Test protocol summary

**Note:** If instructions in this protocol have been followed, samples have been diluted 1:2 (50  $\mu$ L sample + 50  $\mu$ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor ( $\times 2$ ).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100  $\mu$ L Sample Diluent, in duplicate, to all standard wells. Pipet 100  $\mu$ L prepared standard into the first wells and create standard dilutions by transferring 100  $\mu$ L from well to well. Discard 100  $\mu$ L from the last wells.

Alternatively, external standard dilution in tubes (see “External standard dilution” on page 3): Pipet 100  $\mu$ L of these standard dilutions in the microwell strips.

4. Add 100  $\mu$ L Sample Diluent, in duplicate, to the blank wells.
5. Add 50  $\mu$ L Sample Diluent to sample wells.
6. Add 50  $\mu$ L sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50  $\mu$ L Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18–25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100  $\mu$ L diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18–25°C).
14. Empty and wash microwell strips 3 times with Wash Buffer.
15. Add 100  $\mu$ L of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18–25°C).
17. Add 100  $\mu$ L Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

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