Quantikine™ HS ELISA

Human IL-8/CXCL8 Immunoassay

Catalog Number HS800
SS800
PHS800

For the quantitative determination of human Interleukin 8 (IL-8) concentrations in serum and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Interleukin-8 (IL-8), also known as CXCL8, GCP-1, and NAP-1, is a heparin-binding 8-9 kDa member of the alpha, or CXC family of chemokines. There are at least 15 human CXC family members that all adopt a three β-sheet/one α-helix structure. Most CXC chemokines show an N-terminal Glu-Leu-Arg (ELR) tripeptide motif (1, 2). CXCL8 circulates as a monomer, homodimer, and heterodimer with CXCL4/PF4. The monomer is considered the most bioactive, while the heterodimer can potentiate PF4 activity (3-6). CXCL8 oligomerization is modulated by its interactions with matrix and cell surface glycosaminoglycans (GAGs) (7, 8). Mature human CXCL8 shares 65%-69% amino acid (aa) identity with canine, feline, and porcine CXCL8 (9, 10). There is no CXCL8 gene counterpart in rodent.

Multiple isoforms of CXCL8 are generated through both alternate splicing and differential proteolytic cleavage. In humans, alternate splicing generates an isoform with an eleven aa substitution at the C-terminus (11). Proteolytic processing results in N-terminal truncation of CXCL8 and is likely a cell-specific event. For example, fibroblasts and endothelial cells generate the 1-77 aa form by cleaving CXCL8 following Glu21, while monocytes and lymphocytes generate the 6-77 aa form by cleaving following Leu25. These truncated forms generally show increased bioactivity, particularly through the CXCR1 receptor (12-14). CXCL8 can also undergo citrullination on Arg27 of the precursor, a modification that increases its half-life and ability to induce leukocytosis (15, 16). A wide variety of cells secrete CXCL8 including monocytes and neutrophils (17), fibroblasts and keratinocytes (18), mast cells (19), visceral smooth muscle cells (20), dendritic cells (21), type II great alveolar cells (22), and endothelial cells (23).

CXCL8 bioactivity is mediated through two G-protein-coupled receptors, termed CXCR1/IL-8 RA and CXCR2/IL-8 RB (24). CXCR1 is 45-50 kDa in size and is used almost exclusively by CXCL8. CXCR2 is 35-40 kDa in size and is used by nearly all CXC chemokines (25, 26). Both CXCR1 and CXCR2 constitutively associate into functional homodimers. They can also heterodimerize, but these complexes dissociate following CXCL8 binding (27). CXCR2 responds to low concentrations of CXCL8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, in contrast, responds to high concentrations of CXCL8 and is associated with respiratory burst and phospholipase D2 activation (26). Thus, CXCR2 ligation induces leukocyte adhesion to activated vascular endothelium and migration to sites of inflammation, while CXCR1 ligation primes neutrophil antimicrobial activity (28). CXCL8 can also form a complex with Serpin A1/alpha-1 Antitrypsin, and this prevents CXCL8 interaction with CXCR1 (29).

In addition to its pro inflammatory effects, CXCL8 is involved in angiogenesis and the pathogenesis of atherosclerosis and cancer (30-33). It induces VEGF expression in vascular endothelial cells and functions as an autocrine factor for EC growth and angiogenesis (34, 35). It is upregulated in atherosclerotic lesions and is elevated in the serum and cerebrospinal fluid following myocardial infarction (36, 37). In cancer, CXCL8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (32, 38-40).

The Quantikine™ HS Human IL-8/CXCL8 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-8 levels in serum and plasma. It contains *E. coli*-expressed recombinant human IL-8 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human IL-8 accurately. Results obtained using natural human IL-8 showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-8.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-8 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of IL-8 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # HS800	CATALOG # SS800	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-8 HS Microplate	894082	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-8.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-8 HS Conjugate	894083	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-8, conjugated to alkaline phosphatase with preservatives.	Store for up to 1 month at 2-8 °C.*
Human IL-8 HS Standard	894084	2 vials	12 vial	Recombinant human IL-8 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-85	895877	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6Z	895466	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer 10X Concentrate	895188	1 vials	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2N sulfuric acid.	
Substrate	895884	1 vial	6 vials	Lyophilized NADPH with stabilizers.	**Store in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	1 vial	6 vials	7 mL/vial of buffered solution with stabilizers and preservative.	
Amplifier	895886	1 vial	6 vials	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	1 vial	6 vials	7 mL/vial of buffered solution containing INT-violet with stabilizer and preservative.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

HS800 contains sufficient materials to run an ELISA on one 96 well plate.
SS800 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PHS800). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IL-8 HS Microplate	894082	50 plates
Human IL-8 HS Conjugate	894083	50 vials
Human IL-8 HS Standard	894084	100 vials
Assay Diluent RD1-85	895877	50 vials
Calibrator Diluent RD6Z	895466	50 vials
Wash Buffer 10X Concentrate	895188	12 bottles
Stop Solution	895032	50 vials
Substrate	895884	50 vials
Substrate Diluent	895885	50 vials
Amplifier	895886	50 vials
Amplifier Diluent	895887	50 vials
Plate Sealers	N/A	200 sheets
Package Inserts	752194	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, with the correction wavelength set at 650 nm or 690 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser or autowasher
- 1000 mL graduated cylinder for preparation of Wash Buffer
- Test tubes for dilution of standards
- Human IL-8 HS Controls (optional; R&D Systems®, Catalog # QC41)

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Samples allowed to clot longer than 2 hours result in increased IL-8 levels.*

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).*

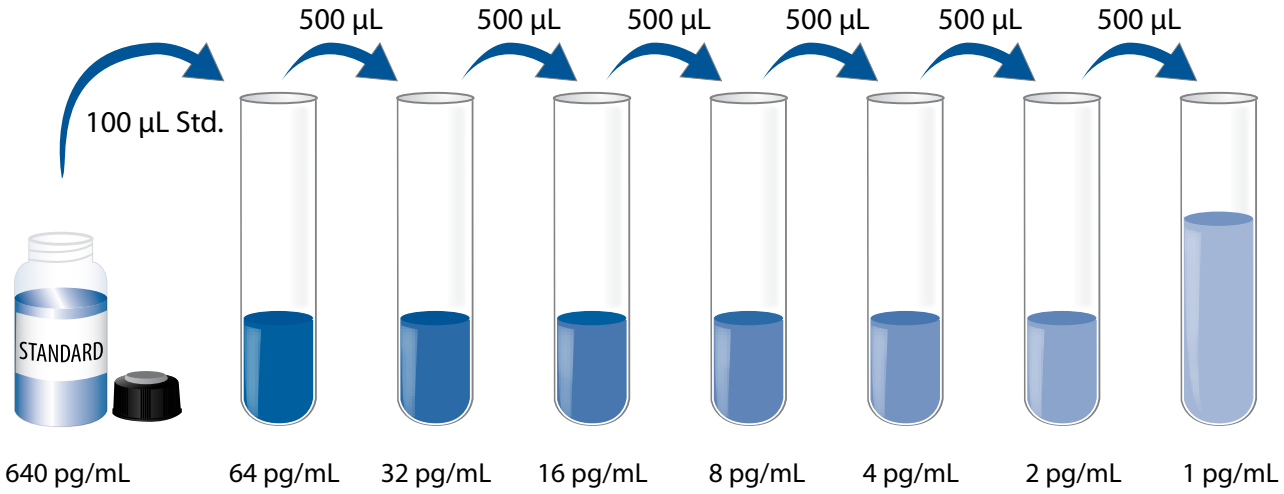
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer 10X Concentrate to 900 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6.0 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Human IL-8 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-8 HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 640 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD6Z into the 64 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 64 pg/mL standard serves as the high standard. Calibrator Diluent RD6Z serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-85 to each well.
4. Add 100 µL of standard, control, or sample per well. Securely cover with a plate sealer and incubate for 2 hours at room temperature. Gently tap the plate to ensure thorough mixing. A plate layout is provided to record standards and samples assayed.
5. Wash
   a. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
   c. Fill each well with 400 µL of Wash Buffer using a squirt bottle, manifold dispenser, or auto washer.
   d. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   e. Repeat steps b, c, and d 5 times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200 µL of Human IL-8 HS Conjugate to all wells. Securely cover with a plate sealer and incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Securely cover with a plate sealer and incubate for 1 hour at room temperature. **Protect from light. Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Securely cover with a plate sealer and incubate for 30 minutes at room temperature.

   Note: *Addition of Amplifier Solution initiates color development.*
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect color in wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

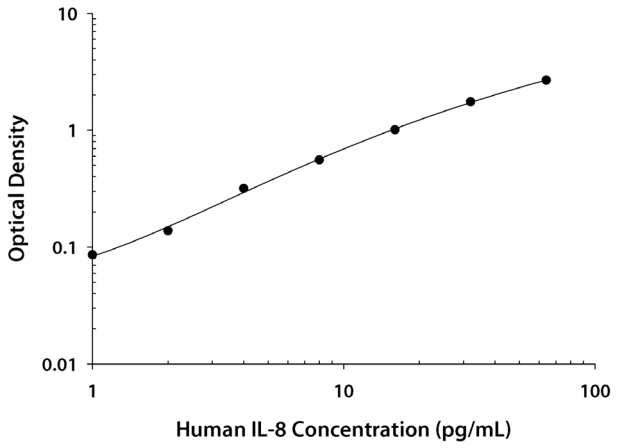
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-8 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.053 0.055	0.054	—
1	0.139 0.141	0.140	0.086
2	0.191 0.194	0.192	0.138
4	0.370 0.373	0.371	0.317
8	0.588 0.634	0.611	0.557
16	1.051 1.074	1.063	1.009
32	1.796 1.816	1.806	1.752
64	2.726 2.743	2.734	2.680

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	5.5	18.7	37.1	5.0	19.4	39.2
Standard deviation	0.3	0.7	2.7	0.4	1.6	3.7
CV (%)	5.5	3.7	7.3	8.0	8.2	9.4

RECOVERY

The recovery of human IL-8 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=4)	99	85-110%
EDTA plasma (n=4)	95	86-102%
Heparin plasma (n=4)	99	91-107%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-8 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	99	103
	Range (%)	98-108	95-105	100-108
1:4	Average % of Expected	103	101	101
	Range (%)	98-114	96-106	89-107
1:8	Average % of Expected	106	107	108
	Range (%)	102-114	100-115	105-113
1:16	Average % of Expected	106	106	103
	Range (%)	101-112	100-115	88-112

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human IL-8 ranged from 0.02-0.40 pg/mL. The mean MDD was 0.13 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed 72 aa (monocyte) form of recombinant human IL-8 produced at R&D Systems®.

The NIBSC/WHO IL-8 1st International Standard 89/520 was evaluated in this kit. The dose response curve of this standard parallels the Quantikine™ HS standard curve. To convert sample values obtained with the Quantikine HS Human IL-8/CXCL8 kit to approximate NIBSC International Units, use the equation below.

NIBSC/WHO (89/520) approximate value (IU/mL) = 0.001 x Quantikine HS IL-8 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-8 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	10.8	2.84-16.5	3.19
EDTA plasma (n=35)	4.39	2.27-11.1	1.63
Heparin plasma (n=35)	6.18	3.53-18.6	2.63

SPECIFICITY

This assay recognizes natural and recombinant human IL-8.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range recombinant human IL-8 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CCL2/MCP-1
CCL23/MPIF-1
CCL27/CTACK
CXCL4/PF4
CXCL12/SDF-1α
CXCL12/SDF-1β
IL-2
IL-13
IL-21
IL-22

Other recombinants:

rat CCL2/JE/MCP-1
feline IL-8/CXCL8
canine IL-8/CXCL8
viral IL-10
viral MIP-1
viral MCV-type II Chemokine-like protein

Recombinant mouse:

CCL2/JE/MCP-1
CXCL1/KC
CXCL4/PF4
CXCL2/MIP-2

Recombinant porcine IL-8/CXCL8 does not interfere but does cross-react approximately 0.32% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.