Quantikine® ELISA

Porcine IL-8/CXCL8 Immunoassay

Catalog Number P8000

For the quantitative determination of porcine Interleukin 8 (IL-8) concentrations in cell culture supernates and serum.

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). IL-8 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). IL-8 oligomerization is modulated by its interactions with matrix and cell surface glycosaminoglycans (GAGs) (7, 8). Mature porcine IL-8 shares 82%, 78%, and 65% aa identity with canine, feline, and human IL-8 (9, 10). There is no IL-8 gene counterpart in rodent.

Proteolytic processing results in N-terminal truncation of IL-8 and is likely a cell-specific event. For example, fibroblasts and endothelial cells generate the 1-77 form by cleaving IL-8 following Glu21, while monocytes and lymphocytes generate the 6-77 form by cleaving following Leu25. These truncated forms generally show increased bioactivity, particularly through the CXCR1 receptor (11-13). IL-8 can also undergo citrullination on Arg27 of the precursor, a modification that increases its half-life and ability to induce leukocytosis (14, 15). A wide variety of cells secrete IL-8 including monocytes and neutrophils (16), fibroblasts and keratinocytes (17), mast cells (18), visceral smooth muscle cells (19), dendritic cells (20), type II great alveolar cells (21), and endothelial cells (22).

IL-8 bioactivity is mediated through two G-protein-coupled receptors, termed CXCR1/IL-8 RA and CXCR2/IL-8 RB (23). CXCR1 is 45-50 kDa in size and is used almost exclusively by IL-8. CXCR2 is 35-40 kDa in size and is used by nearly all CXC chemokines (24, 25). Both CXCR1 and CXCR2 constitutively associate into functional homodimers. They can also heterodimerize, but these complexes dissociate following IL-8 binding (26). CXCR2 responds to low concentrations of IL-8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, in contrast, responds to high concentrations of IL-8 and is associated with respiratory burst and phospholipase D2 activation (25). Thus, CXCR2 ligation induces leukocyte adhesion to activated vascular endothelium and migration to sites of inflammation, while CXCR1 ligation primes neutrophil antimicrobial activity (27). IL-8 can also form a complex with Serpin A1/alpha-1 Antitrypsin, and this prevents IL-8 interaction with CXCR1 (28).

In addition to its pro-inflammatory effects, IL-8 is involved in angiogenesis and the pathogenesis of atherosclerosis and cancer (29-32). It induces VEGF expression in vascular endothelial cells and functions as an autocrine factor for EC growth and angiogenesis (33, 34). It is upregulated in atherosclerotic lesions and is elevated in the serum and cerebrospinal fluid following myocardial infarction (35, 36). In cancer, IL-8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (31, 37-39).

The Quantikine® Porcine IL-8/CXCL8 Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-8 in cell culture supernates and serum. It contains *E. coli*-expressed recombinant porcine IL-8 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant porcine IL-8. Results obtained using natural porcine IL-8 show dose response curves that are parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural porcine IL-8.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for porcine IL-8 has been pre-coated onto a microplate. Standards, control, 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 porcine 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. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-8 bound in the initial step. The sample values are then read off the standard curve.

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 the calibrator diluent and repeat the assay. If cell culture supernate samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with calibrator diluent.
- Any variation in standard 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

- When mixing or reconstituting 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Porcine IL-8 Microplate	892182	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for porcine 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.*
Porcine IL-8 Standard	892184	2 vials of recombinant porcine IL-8 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay.
Porcine IL-8 Control	892185	2 vials of recombinant porcine IL-8 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Porcine IL-8 Conjugate	892183	21 mL of a polyclonal antibody specific for porcine IL-8 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-40	895513	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	21 mL of diluted animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.

PRECAUTIONS

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.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS 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.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

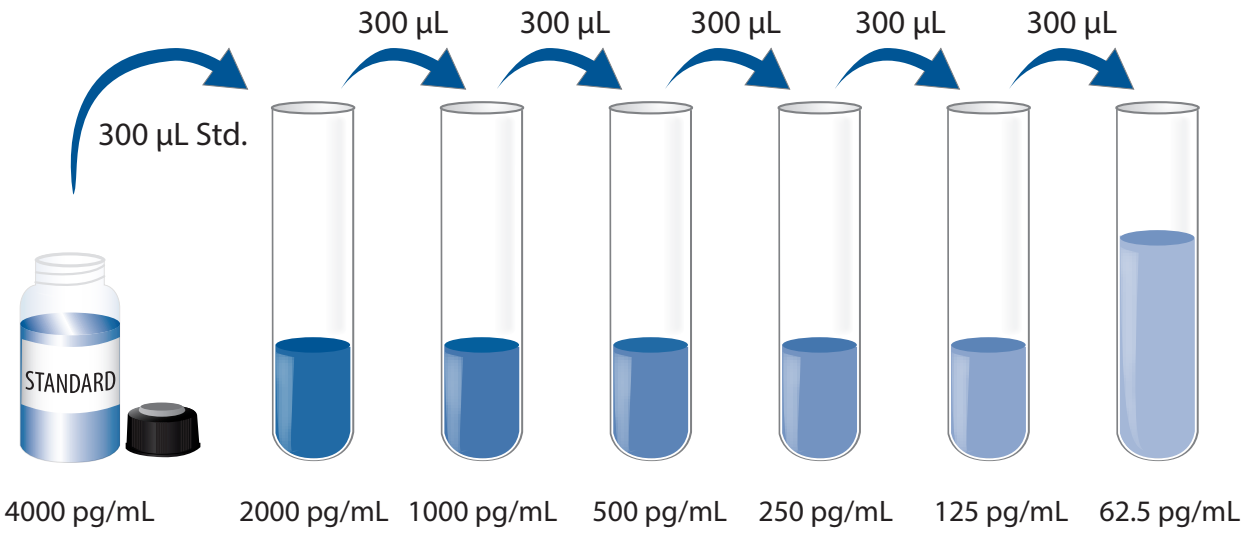
Porcine IL-8 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 120 µL of the resultant mixture is required per well.

Porcine IL-8 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Porcine IL-8 Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 300 µL of Calibrator Diluent RD5-16 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Porcine IL-8 Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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 samples, standards, and control be assayed in duplicate.

1. Prepare reagents, samples, control, and standard dilutions as directed by 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 50 µL of Assay Diluent RD1-40 to each well.
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Porcine IL-8 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 120 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

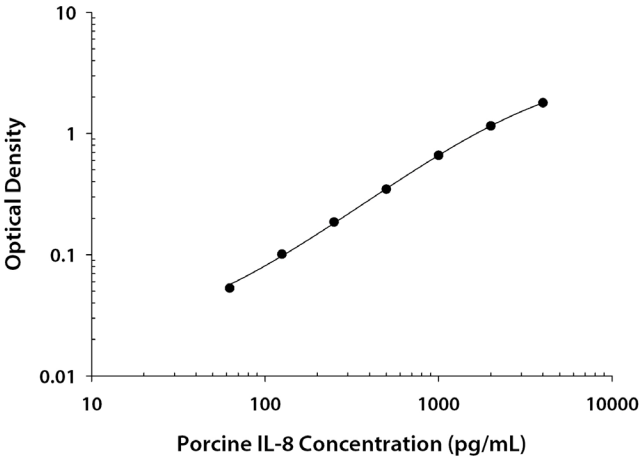
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 porcine 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	0.083 0.083	0.083	—
62.5	0.133 0.140	0.136	0.053
125	0.178 0.191	0.184	0.101
250	0.267 0.271	0.269	0.186
500	0.418 0.443	0.430	0.347
1000	0.717 0.772	0.744	0.661
2000	1.235 1.243	1.239	1.156
4000	1.865 1.885	1.875	1.792

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	97	748	1607	106	717	1641
Standard deviation	9.4	64	106	9.0	49	108
CV (%)	9.7	8.6	6.6	8.5	6.8	6.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernate (n=7)	108	80-120%
Serum (n=7)	91	80-100%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of porcine IL-8 in each matrix were diluted with calibrator diluent and assayed.

		Cell culture supernates (n=2)	Spiked Cell culture media (n=4)	Serum (n=5)
1:2	Average % of Expected	108	100	100
	Range (%)	105-111	92-114	90-112
1:4	Average % of Expected	106	91	100
	Range (%)	99-112	87-99	89-108
1:8	Average % of Expected	104	90	100
	Range (%)	91-117	83-95	93-111
1:16	Average % of Expected	108	95	102
	Range (%)	———	88-106	90-114

SENSITIVITY

Thirteen assays were evaluated and the minimum detectable dose (MDD) of porcine IL-8 ranged from 1.0-6.7 pg/mL. The mean MDD was 4.6 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 recombinant porcine IL-8 produced at R&D Systems®.

SAMPLE VALUES

Serum - Eight samples were evaluated for detectable levels of porcine IL-8. Five samples read below the lowest standard, 62.5 pg/mL. The remaining three samples read 179 pg/mL, 131 pg/mL, and 96 pg/mL.

Cell Culture Supernates - Porcine peripheral blood leukocytes (5 x 10^6 cells/mL) were cultured in DMEM with 10% fetal bovine serum unstimulated and stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin for 48 hours. Aliquots of the cell culture supernates were removed, assayed for porcine IL-8, and measured 7980 pg/mL and 59,040 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant porcine 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 50 ng/mL in a mid-range porcine IL-8 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:

GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-4
IL-6
IL-10
IL-12
PDGF
TGF-β1
TNF-α

Recombinant human:

IL-8

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