Human IL-8 ELISA Kit

Catalog Number：RK00011

This ELISA kit used for quantitative determination of human interleukin 8 (IL-8) concentrations in cell culture supernates, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

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Introduction

Interleukin-8 (IL-8), also known as IL-8, 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 bio-active, 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 human IL-8 shares 65-69% amino acid (aa) identity with canine, feline, and porcine IL-8 (9, 10). There is no IL-8 gene counterpart in rodent.

Multiple isoforms of IL-8 are generated through both alternative splicing and differential proteolytic cleavage. In humans, alternative splicing generates an iso-form with an eleven aa substitution at the C-terminus (11). 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 (12-14). IL-8 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 IL-8 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). IL-8 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 IL-8. 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 IL-8 binding (27). 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 (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). IL-8 can also form a complex with Serpin A1/alpha-1 Antitrypsin, and this prevents IL-8 interaction with CXCR1 (29).

In addition to its pro-inflammatory effects, IL-8 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, IL-8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (32, 38-40).

Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IL-8 is added to the wells and binds to the combination of capture antibody-IL-8 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of IL-8 present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven IL-8 standard dilutions and IL-8 sample concentration determined.

Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	8×12	RM00072	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C.Reseal along entire edge of zip-seal.
Standard Lyophilized	2	RM00069	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (100×)	1 ×120ul	RM00070	May be stored for up to 6 month at 2-8 °C.*
Streptavidin-HRP Concentrated (40×)	1 ×300ul	RM00071	
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate Antibody Diluent (R2)	1 ×12mL	RM00024	
Streptavidin-HRP Diluent(R3)	1 ×12mL	RM00025	
Wash Buffer(20x)	1 × 30mL	RM00026	
TMB Substrate	1 ×12 mL	RM00027	
Stop Solution	1 ×6 mL	RM00028	
Plate Sealers	4 strips		
Specification	1		

Sample Collection And Storage

1. **Cell Culture Supernates:**

   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

2. **Serum:**

   Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

3. **Plasma**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**

5. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.

17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

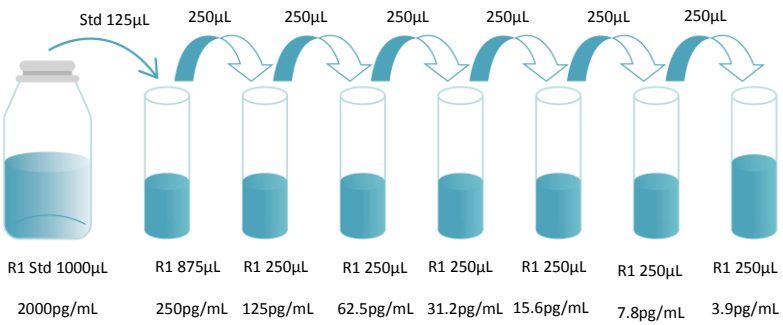
Experiment Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 μL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**

2. **Standard:** Add Standard/Sample Diluent(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (2000 pg/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 250, 125, 62.5, 31.25, 15.6 7.8, 3.9, 0 pg/mL). Redissolved standard solution (2000 pg/mL), aliquot and store at -20°C— -70°C.



3. **Concentrated Biotin Conjugate Antibody (100x)** : Dilute 1:100 with the Biotin-Conjugate Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

4. **Streptavidin-HRP Concentrated (40x):** Dilute 1:40 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Streptavidin-HRP (40x)	Streptavidin-HRP Diluent(R3)
2	50uL	1950uL
4	100uL	3900uL
6	150uL	5850uL
8	200uL	7800uL
10	250uL	9750uL
12	300uL	11700uL

5. **Wash buffer**: Dilute 1:20 with double distilled or deionized water before use.

Wash Method

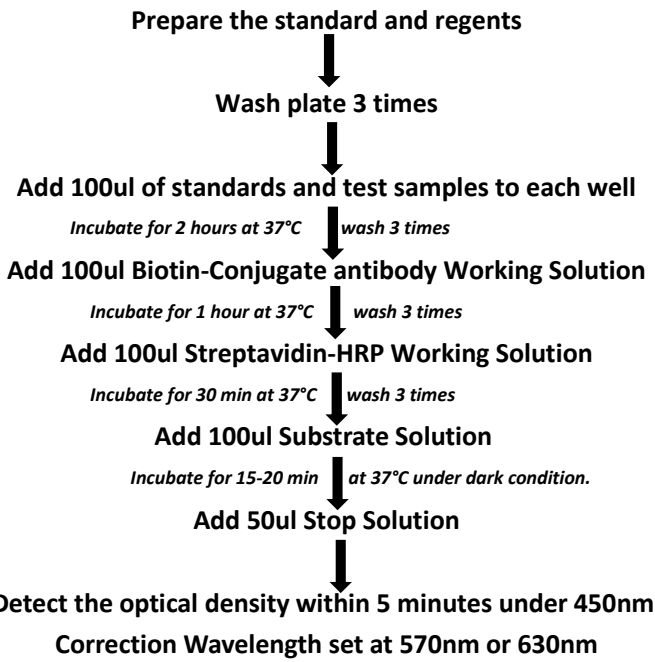
Aspirate each well and wash, repeating the process two times for a total of three washes.Wash by filling each well with **Wash Buffer**(300ul) using a squirt bottle,manifold dispenser,or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash,remove any remaining **Wash Buffer** by aspirating or decanting.Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100 µL Standard/sample Diluent (R1) in blank well.
4. Add 100 µL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Biotin Conjugate Antibody (100X) Working Solution 15 minutes early before use.
7. Add Biotin-Conjugate Antibody Diluent(R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
8. Prepare the Streptavidin-HRP Concentrated (40X) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 2.
10. Add Streptavidin-HRP Diluent(R3) in blank well and add Streptavidin-HRP Working Solution in other wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
11. Warm-up the Microplate reader.
12. Repeat the aspiration/wash as in step 2.
13. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
14. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength

correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 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.

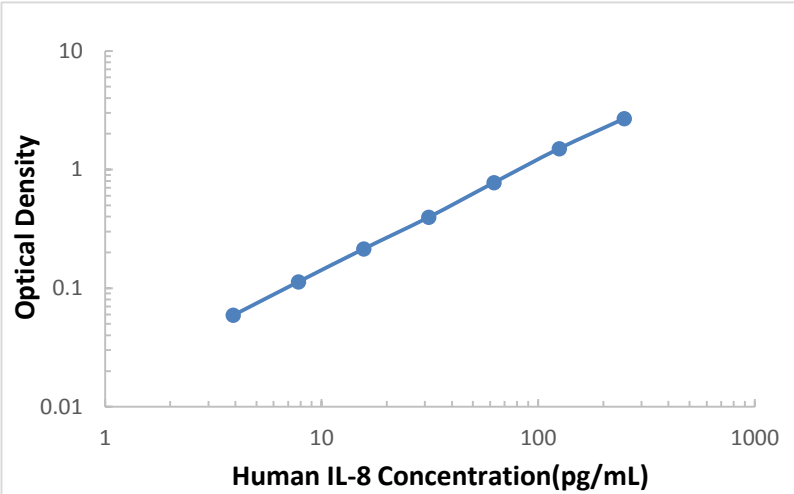
Assay Procedure Summary



Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the IL-8 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of IL-8 assayed.

Sensitivity

The minimum detectable dose (MDD) of IL-8 is typically less than 6.2 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural human IL-8. The factors listed below were prepared at 50ng/ml and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

Recombinant human	Recombinant mouse	Recombinant canine cytokines
ENA-78	BLC/BCA-1	IL-8
BLC/BCA-1	GCP-2	
GCP-2	CRG-2	
GROα	KC	
GROβ	MIG	
GROγ	SDF-1β	
IP-10		
I-TAC		
MIG		
NAP-2		
PF4		
SDF-1α		
SDF-1β		

Precision

Intra-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	85.0	549	1068
Standard Deviation (SD)	3.8	21.4	44.9
Variable Coefficient CV (%)	4.5	3.9	4.2

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	84.5	916	1960
Standard Deviation (SD)	6.4	72.4	147
Variable Coefficient CV (%)	7.6	7.9	7.5

Recovery

Spike 3 different concentration of human IL-8 into healthy human serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	97	92-101
Plasma	103	94-112

Linearity

Spike high concentration of human IL-8 into 4 healthy human serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	102	95-108
1:4	97	89-105
1:8	98	93-102
1:16	99	93-104

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