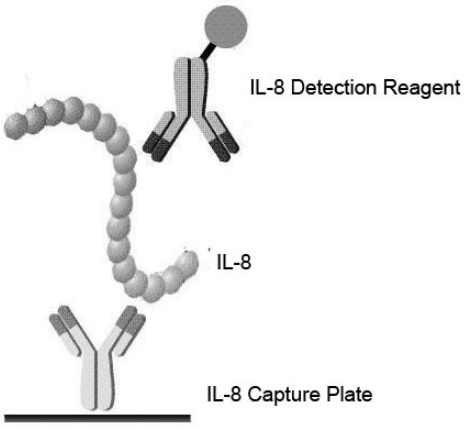
Human IL-8 ELISA Detection Kit **Cat. No. L00417**

Technical Manual No. 0603 **Version 05142012**



Genscript Human IL-8 ELISA Kit is for the quantitative determination of human IL-8 in serum, plasma and cell culture supernatant.

The operator should read technical manual carefully before using this product.
For research use only. Not for use in diagnostic procedures.

Contents

I. Description

Interleukin-8 (IL-8), a member of a family of proinflammatory cytokines, is a neutrophil chemotactic cytokine that can signal through the CXCR1 and CXCR2 receptor. It is proinflammatory and primarily mediates the activation and migration of neutrophils from peripheral blood into the sites of inflammation, injury, or infection in the tissue. IL-8 is involved in a wide variety of physiological and pathological processes, including host defense against bacterial infection, bronchiolitis, arteriosclerosis, autoimmune disorders of skin, and angiogenesis-dependent disorders such as tumor growth, and wound repair. IL-8 is produced by stimulated monocytes, leukocytic cells, non-leukocytic somatic cells and tumor cells.

GenScript Human IL-8 ELISA Detection Kit can be applied for quantitative detection of Human IL-8 concentrations in plasma, blood and cell culture supernatant samples. The assay utilizes solid sandwich enzyme immunoassay in which two anti IL-8 monoclonal antibodies function as bridge linkers. IL-8 Capture Plate, which is a 96-well plate pre-coated with one anti IL-8 monoclonal antibody, is used for capture of IL-8 in serial IL-8 standard solutions and sample solutions. IL-8 Detection Reagent, which is a horseradish peroxidase (HRP) enzyme conjugated to the other anti IL-8 monoclonal antibody, is used for enzyme reaction. In the assay, IL-8 molecular is fixed between the Capture Plate and the Detection Reagent to form a sandwich complex vial both antibodies. Other unbound moleculars can be removed by several washings. The IL-8 Detection Reagent reacts with TMB substrate that provides absorbance value, which can be obtained by Microplate reader. In optimized test condition, each absorbance value is indicated to the individual IL-8 amount in solution. The IL-8 standards of known concentration and corresponding absorbance values are used to form a standard curve. With the standard curve, IL-8 amount present in the unknown sample is calculated by transforming its absorbance value.

II. Key Features

- Sensitivity: 2.8 pg/ml
- Detection Range: 25～1600 pg/ml
- Test Samples: serum, plasma and cell culture supernatant
- Time Saving Protocol - complete test in 2.5 hours
- Ready-to-use ELISA plate with fast & simple sample preparation
- Lot-to-lot consistency guaranteed by strict procedure

III. Kit Contents

The kit provides all components necessary for human IL-8 quantification. Sufficient materials are provided to yield to test one plate in this package.

Components	Quantity	Part.No
IL-8 Capture Plate	1 plate (8 wells x 12 strips)	417-80
IL-8 Detection Reagent	15 ml	417-30
IL-8 Standard	2 vials	417-10
Sample Diluent	30 ml	417-60
Assay Diluent	15 ml	417-90
20 × Wash Solution	40 ml	417-70
TMB Substrate	12 ml	417-40
Stop Solution	6 ml	417-50
Adhesive Plate Cover	2 pieces	N/A
Technical Manual	1 copy	N/A

IV. Storage

The reagents in the kit are stable for 12 months when stored at 2-8°C. It should not be used beyond the expiration date. Do not freeze the kit.

V. Reagents/Equipments Required But Not Supplied

Microplate reader capable of measuring absorbance at 450 nm

Automated microplate washer

Deionized or distilled water

Graduated cylinder to prepare Wash Solution

Plastic container to prepare Wash Solution

Tubes to prepare standard dilutions and to aliquot samples

Precision pipettes to deliver 10 µl, 100 µl, 200 µl and 1000 µl content

10 µl, 100 µl, 200 µl and 1000 µl pipette tips

Multichannel pipettor

Disposable reagent reservoirs

Paper towel

Laboratory timer

Refrigeratory to store samples and kit components

VI. Instruction for Use

Test Sample Preparation

- Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood-bore infection.
- When the expected IL-8 concentration in a sample exceeds 1600 pg/ml (the highest concentration of standard curves), dilute the sample with the Sample Diluent prior to performing the assay. Generally cell culture supernatant should be diluted.

Cell culture supernatant: Centrifuge the sample to remove the particulate materials. Run the assay immediately, otherwise aliquot and store sample below -20°C. Avoid repeat thaw-freeze cycle.

Serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Run assay immediately, otherwise aliquot and store sample below -20°C. Avoid repeat thaw-freeze cycle.

Plasma: Treat blood with citrate, EDTA or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes for plasma collection. Then Run assay immediately. Otherwise aliquot and store sample below -20°C. Avoid repeat thaw-freeze cycle.

Reagent Preparation

- If any precipitate is found in the 20 × Wash Solution, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate disappears.

Wash Solution: Add 40 ml of 20 × Wash Solution to 760 ml of deionized or distilled water to make 800 ml of Wash Solution. Store the diluted Wash Solution at 2-8°C.

IL-8 Standard Preparation:

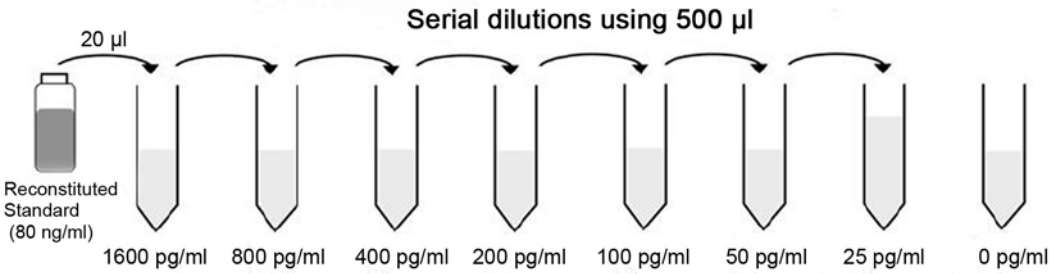
- Allow IL-8 stock solution to sit for a minimum of 15 minutes at room temparature with gentle agitation prior to making dilution.
- Store the IL-8 stock solution below -20°C. Avoid repeat thaw-freeze cycle.

Reconstitute IL-8 Standard by adding 500 µl of deionized water (or distill water). This reconstitution provides the IL-8 stock solution with the IL-8 concentration of 80 ng/ml.

Prepare IL-8 standard curves for example:

1. Label eight 1.5 mL Eppendorf tubes with '1600 pg/ml', '800 pg/ml', '400 pg/ml', '200 pg/ml', '100 pg/ml', '50 pg/ml', '25 pg/ml' and '0 pg/ml'.
2. Pipette 20 µl of 80.0 ng/ml IL-8 stock solution and 980 µl of Sample Diluent into the tube labeled with '1600 pg/ml' and vortex it.
3. Pipette 500 µl of Sample Diluent into the rest empty tubes.

4. Pipette 500 µl of 1600 pg/ml IL-8 solution to the tube labeled with '800 pg/ml' and vortex it to make the standard point of 800 pg/ml.
5. Similarly, prepare of rest standard points in the standard series (400, 200, 100, 50, 25 pg/ml).



Microtiter Plate Preparation

- It is recommended that all standards and samples are prepared in duplicate.
- Make sure the strips are tightly snapped in the plate frame.

1. Count the strips for assay.
2. Leave the unused strips in the foil pouch and store at 2-8°C.

Test Procedure

- Reagents and samples should be fully equilibrated to room temperature (20-25°C) before performing a test. Do not use heated water baths to thaw or warm samples.
- When covering the plate with adhesive plate cover, press your fingers over the plate edges and down each strip to ensure a tight seal of the plate.
- Calculate the reaction time with a Laboratory timer.

Standards and Samples Incubation

1. Pipette 100 µl of Assay Diluent to each well of the plate.
2. Pipette 100 µl of prepared Human IL-8 standards and test samples to different wells.
3. Cover the plate with adhesive plate cover and incubate at 4°C for an hour.
4. Remove the adhesive plate cover and aspirate the solution from the wells.
5. Wash each well of the plate with 260 µl of prepared Wash Solution for four times.
6. Invert the plate and pound it vigorously on clean paper towels to remove excess liquid in each well.

IL-8 Detection Reagent Incubation

- Briefly vortex bottle containing IL-8 Detection Reagent before use.

7. Pipette 100 µl *of* IL-8 Detection Reagent to each well.
8. Cover the plate with adhesive plate cover and incubate the plate at 4°C for an hour.
9. Remove the adhesive plate cover and aspirate the solution from the wells.
10. Wash the plate with 260 µl of prepared Wash Solution for four times.
11. Invert the plate and pound it vigorously on clean paper towels to remove excess liquid in each well.

Substrate Reaction and Absorbance Measurement

- Does not combine the unused TMB Substrate and that preserved in bottle for leftover strips.
- The actual reaction time depends on the temperature. If temperature is low, reaction time should be extended. The ideal reaction temperature for optimum assay performance is 20‐25°C.
- Reliable standard curve is obtained when the OD_{450} absorbance value does not exceed 0.1 unit for the zero standard concentration.

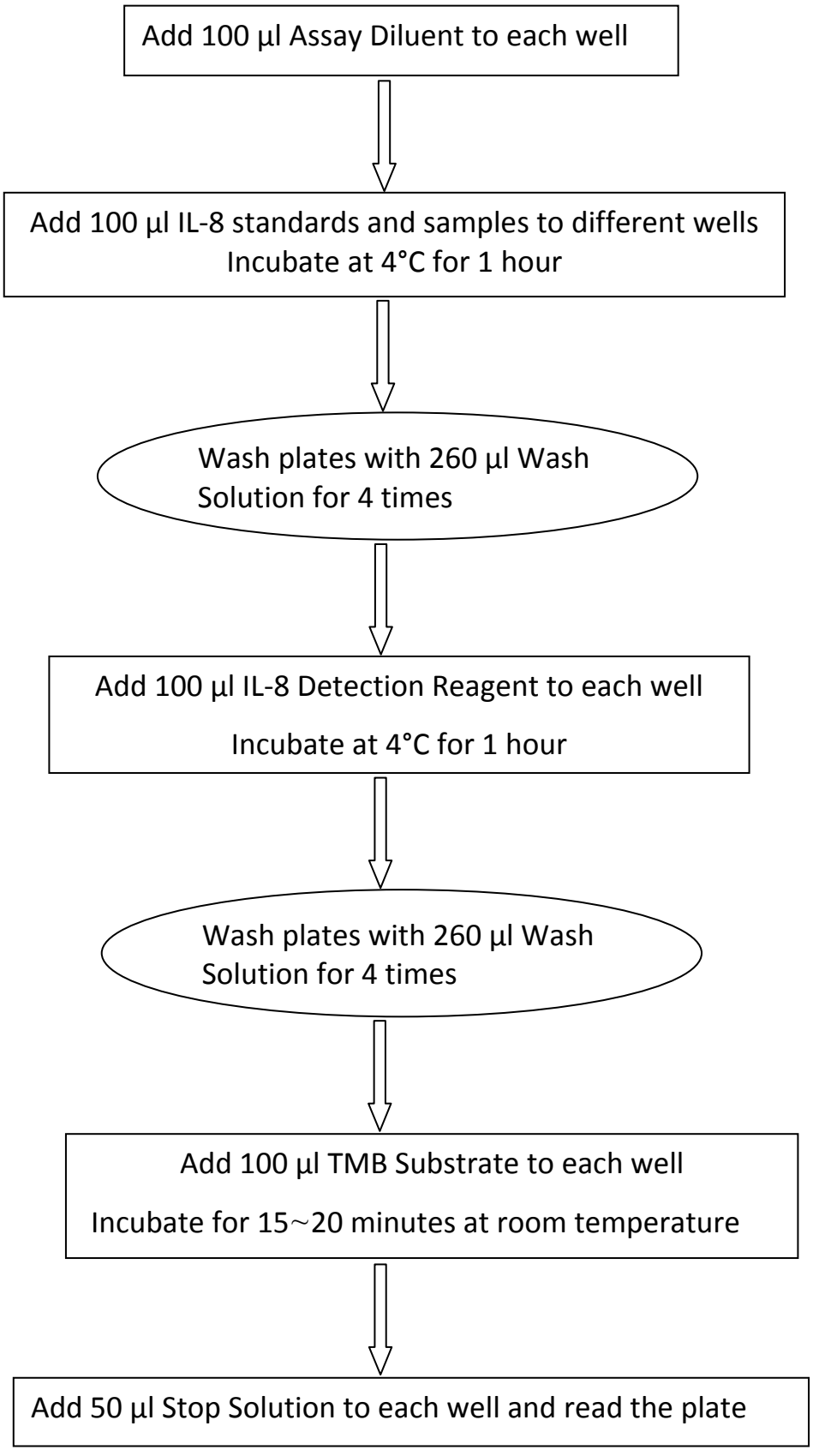
12. Transfer certain TMB Substrate to a disposable reagent reservoir.
13. Pipette 100 µl of TMB Substrate from the reagent reservoir to each well.
14. Incubate at room temperature for 15～20 minutes in darkness environment.
15. Transfer certain Stop Solution to a new disposable reagent reservoir.
16. Pipette 50 µl of Stop Solution to each well to stop the reaction.
17. Read the plate at 450nm with a Microplate reader to obtain absorbance values.

IL-8 Amount Calculation

- If the sample is diluted, multiply the interpolated value by the dilution factor to calculate pg/ml of human IL-8 in the sample.

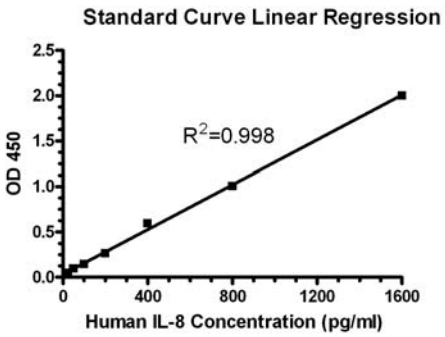
18. Calculate the mean absorbance for each set of duplicate standards and samples.
19. Subtract the zero mean absorption from each mean absorbance to receive adjusted absorbance value.
20. Prepare a standard curve with linear regression method in statistical software. Plot the standard curve with serial standards (pg/ml) on the x-axis versus the corresponding subtracted absorbance values on the y-axis.
21. Determine the IL-8 amount in sample by interpolating from its adjusted absorbance value to IL-8 concentration with the standard curve.

VII. Assay Procedure Summary



VIII. Typical Assay Data

The standard curve was provided for demonstration only. It should be prepared each time an assay is performed.



Conc. of Human IL-8 (pg/ml)	OD_{450}			
	Duplicate 1	Duplicate 2	Average	Adjusted Average
1600	2.104	2.050	2.077	2.001
800	1.095	1.072	1.084	1.008
400	0.698	0.646	0.672	0.596
200	0.334	0.345	0.339	0.263
100	0.220	0.222	0.221	0.145
50	0.171	0.169	0.170	0.094
25	0.121	0.124	0.123	0.047
0	0.077	0.075	0.076	-

IX. Precision

Intra-assay: Three different levels of Human IL-8 were tested 20 times on one plate to assess intra-assay precision.

Inter-assay: Three different levels of Human IL-8 were tested in 20 separate assays to assess inter-assay precision.

Intra-assay				Inter-assay			
# of replicates	Mean (pg/ml)	SD	CV%	# of replicates	Mean (pg/ml)	SD	CV%
20	172	7.4	4.3	20	165	12.1	7.3
20	496	25.3	5.1	20	473	37.8	7.9
20	1080	57.2	5.3	20	1143	95.8	8.4

X. Sensitivity

The minimum detectable dose (MDD) of IL-8 was 2.8 pg/ml. MDD is defined as the concentration corresponding to a signal three standard deviations above the mean of the zero standard.

XI. Recovery

Three different levels of IL-8 were spiked into samples of various matrices to assess the recovery of the assay.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=6)	97	87-113
Serum (n=6)	99	92-108
EDTA plasma (n=6)	105	95-109
Heparin plasma (n=6)	101	94-118
Citrate plasma (n=6)	96	91-112

XII. Linearity

Samples spiked with high concentrations of IL-8 were serially diluted with Sample Diluent to assess the linearity of the assay.

Dilution		Cell culture Media (n=5)	Serum (n=5)	EDTA Plasma (n=5)	Heparin Plasma (n=5)	Citrate Plasma (n=5)
1:2	Average % of Expected	93	94	94	96	92
	Range (%)	91-99	91-97	90-96	87-108	89-95
1:4	Average % of Expected	99	98	98	94	92
	Range (%)	97-104	90-105	89-105	89-97	88-100
1:8	Average % of Expected	103	96	93	93	94
	Range (%)	98-110	92-112	91-110	86-102	91-103
1:16	Average % of Expected	107	102	95	92	91
	Range (%)	92-111	89-107	88-104	91-99	84-110

XIII. Specificity

This assay could detect both natural and recombinant human IL-8. The factors listed below were prepared at 50 ng/mL in Sample Diluent and it showed no cross-reactivity with other human factors: IL-1α, IL-1β, IL-4, IL-6, IL-18, IP-10, IGF-1, IGF-2, IFN-γ, IGF-BP4, MCP-1, GM-CSF, SCF, CT-1, FGF-acidic, FGF-6, FGF-16, FGF-17, WISP, Leptin, TNF, Trx, Endostatin, EGF).

XIV. Calibration

This immunoassay is calibrated against purified bacteria expressed recombinant Human IL-8 produced at Genscript company.The NIBSC/WHO International standard 89/520 was evaluated in this kit. The conversion factor for NIBSC material is as follows:

NIBSC 89/520 approximate value (U/ ml) =0.01X Genscript IL-8 value (pg/ml)

XV. Expected Values

Serum/Plasma:

Apparently healthy normal donor's plasma and serum samples were tested in this assay. IL-8 amounts in all tested samples were measured less than the lowest IL-8 standard (25 pg/ml). No medical histories were available for the donors used in this study.

Cell culture supernatant:

Human peripheral blood mononuclear cells from apparently healthy, normal donors were cultured in RPMI-1640 cell culture with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin sulfateand stimulated with 10 µg/ml PHA. Aliquots of the culture supernate were removed on days 1 and 6 and assayed for levels of natural IL-8. The culture supernatant samples were diluted 100-fold in the Sample Diluent.

Condition	Day 1 (pg/ml)	Day 6 (pg/ml)
Unstimulated	11,000	29,000
Stimulated	54,000	93,000

XVI. Troubleshooting

Problem	Probable Cause	Solution
Poor Precision	Omission of adding Assay Diluent	Follow the manual to repeat assay
	Wells were not washed or aspirated properly	Make sure the wash apparatus work properly and wells are dry after aspiration
	Wells have been scratched with pipette tip or washing needles.	Dispense and aspirate solution into and out of wells with caution
	Particulates were found in the samples	Remove any particulates by centrifugation prior to the assay
	Hemolysis in blood sample	Prepare new samples
Poor Standard Curve	Improper preparation of standards	Prepare new Standards as the manual described
	Wells were not washed or aspirated properly	Make sure the wash apparatus work properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components were used from other lots or sources	Never substitute any components from another kit
	Components were not brought to room temperature prior to assay.	Repeat assay with components that have been equilibrated to room temperature
	Incubation steps were performed at wrong temperatures	Perform incubation step as the manual describes
Weak/No Signal	TMB Substrate were not added or were added at the wrong time	Follow the Manual to add the Substrate
	IL-8 Detection Reagent was not added, or was added at the wrong time	Follow the manual to repeat the assay
	Components were used from other lots or sources	Use only lot-specific components
	TMB Substrate has been contaminated	Use new TMB Substrate
	Did not add the proper volumes of reagents	Repeat assay with the required volumes in manual
	Did not incubate the plate for proper time or temperature	Follow the manual to repeat assay
	Did not read the plate immediately after Stop Solution was added	Read the plate within 30 minutes
High Background	Plate was not washed properly	Make sure the wash apparatus is functioning properly. Make sure all wash buffers is removed before adding substrate
	TMB Substrate has been contaminated	Use new TMB Substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with Adhesive Plate Cover in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
	TMB Substrate exposed to light	Use new TMB Substrate

XVII. Plate Layout

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes:

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