BioVendor Group

ENG

Instructions for use:

HUMAN INTERLEUKIN-8 ELISA

Catalogue number:

RD194558200R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current Version
ENG.003.A	ENG.004.A
Added the “History of changes”.	
A sentence „**Centrifuge liquid containing microtube vials before opening**“ has been added to Chapter 9.	
Chapter 11, point 11. Version 003.A “Incubate the plate for **10 minutes** at room temperature” Version 004.A “Incubate the plate for **20 minutes** at room temperature”	

1. INTENDED USE

The RD194558200R Human Interleukin-8 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human IL-8.

Features

- It is intended for research use only
- The total assay time is less than 4 hours
- The kit measures IL-8 in serum, plasma (EDTA, heparin, citrate) and saliva
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Interleukin (IL)-8, a member of the C-X-C chemokine subfamily, is a key mediator of inflammation. It is a product of the CXCL8 gene. It is a basic non-glycosylated protein with pI of 8.3. It is heat-stable protein resistant to plasma peptidases and pH extremes (1, 2).
IL-8 is produced by several cell types such as activated monocytes and macrophages, T cells, neutrophils, NK cells and also endothelial cells, wide variety of epithelial cells and fibroblasts (3). Its production is triggered by proinflammatory cytokines, growth factors and pathophysiologic conditions (2).
This protein acts as a chemotactic factor that attracts neutrophils and has a key role in defense mechanism through the effects on neutrophil activity including neutrophil degranulation and respiratory burst (1). It also affects other types of leukocytes, such as T cells, B cells and basophils (4).
IL-8 mediate its biological functions by interacting with two homologous specific G-protein-coupled CXC chemokine receptors (CXCR1 and CXCR2) (5).

IL-8 is generated as a 99 amino acid-precursor and is secreted after cleavage of a signal sequence composed of 20 residues. NH2-terminal extracellular processing of the mature form yields several biologically active variants (1). The predominant variants consist of 77 or 72 amino acids residues (2).
IL-8 contains four cysteines that form two disulphide bridges. When the disulphide bonds are reduced, the protein is rapidly inactivated (1). IL-8 forms homodimers. The dimer consists of two antiparallel alpha-helices lying on top of a six-stranded antiparallel beta-sheet. However, at nanomolar concentrations, which represent physiologically relevant levels and induce maximal biological activity, most IL-8 occurs in its monomeric form (6, 7).
Because of its potent pro-inflammatory properties, IL-8 is tightly regulated, and its expression is low or undetectable in normal tissues (8). IL-8 molecule is produced in early inflammatory phase and remains active for a long period of time at the site of inflammation. This is in contrast to most of the other inflammatory cytokines, which disappear from the site of inflammation after a few hours in vivo (9). Expression of IL-8 can be induced by IL-1Beta, TNF-α, IL-6, interferon-γ, lipopolysaccharide, phytohemagglutinin, phorbol myristate acetate, reactive oxygen species, and other cellular stresses (10). Potent inhibitors of IL-8 production include dexa-methasone, IL-4, and IL-10 (11).
Increased expression of IL-8 and/or its receptors has been found in cancer cells, endothelial cells, infiltrating neutrophils, and tumor-associated macrophages, suggesting that IL-8 may function as a significant regulatory factor within the tumor microenvironment (12). Experiments also suggest IL-8 is critical to glial tumor neovascularity and progression (13).
Elevated serum and plasma IL-8 levels were observed in women with severe or milder pre-eclampsia of unknown origin compared with normal pregnant women (14, 15).
With regards to the pulmonary diseases, levels of IL-8 are increased in bronchoalveolar lavage fluid (BALF) in acute respiratory distress syndrome patients and correlated with the development of this disease in at-risk patient groups (16). Increased IL-8 expression by alveolar macrophages is also observed in idiopathic pulmonary fibrosis (IPF). IL-8 levels in both serum and BALF are increased significantly, and serum levels are indicative of the disease activity of IPF (17).
Increased levels of IL-8 were observed in serum of critically-ill patients within septic shock onset compared to healthy control. Increasing levels corresponded with sepsis severity (18). IL-8 was also expressed in affected skin of psoriatic patients but not in clinically normal skin of healthy subjects (19).
Regarding other biofluids, it was found that IL-8 is the most abundant cytokine in saliva (20). In gingivial crevicular fluid of periodontitis patients, significantly higher level of total amounts of IL-8 compared to healthy control group was observed (21).

Areas of investigation:
Cytokines
Immune Response, Infection and Inflammation
Oncology
Periodontitis
Sepsis

4. TEST PRINCIPLE

In the BioVendor Human Interleukin-8 ELISA, standards and samples are incubated in microplate wells pre-coated with monoclonal anti-human IL-8 antibody. After 60 minutes incubation and washing, biotin-labelled monoclonal anti-human IL-8 antibody is added and incubated with captured IL-8 for 60 minutes. After another washing, the streptavidin-HRP conjugate is added. After 60 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of IL-8. A standard curve is constructed by plotting absorbance values against concentrations of IL-8 standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5 -1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Centrifuge liquid containing microtube vials before opening.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with dessiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Biotin-Ab Diluent

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

Human IL-8 Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (do not foam).

The resulting concentration of IL-8 in the stock solution is **100 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	–	100 pg/ml
250 µl of stock	250 µl	50 pg/ml
250 µl of 50 pg/ml	250µl	25 pg/ml
250 µl of 25 pg/ml	250 µl	12.5 pg/ml
250 µl of 12.5 pg/ml	250 µl	6.25 pg/ml
250 µl of 6.25 pg/ml	250 µl	3.13 pg/ml
250 µl of 3.13 pg/ml	250 µl	1.56 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted Master Standard and/or diluted standard solutions.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Biotin-Ab Diluent. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. e.g. 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human IL-8 in serum, plasma (EDTA, citrate, heparin) and saliva.

Serum and plasma samples

Samples can be assayed immediately after collection, or should be stored frozen. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples. **Separate the serum or plasma from the cells within two hours**.

- **Dilute serum samples 6x** with Dilution Buffer just prior to the assay. e.g. 25 µl of sample + 125 µl of Dilution Buffer for singlets, or preferably 40 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

- **Dilute plasma samples 3x** with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 80 µl of sample + 160 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Saliva samples

Collect saliva into a clean tube without force or inducement and before eating, drinking or brushing the teeth. Before saliva collection, rinse the mouth with water. Avoid using blood-contaminated specimens. Store samples at -70°C. Just prior to the assay, centrifuge thawed specimens at 13,000 rpm for 5 min. Collect supernatants into freshly labelled tubes.

Dilute saliva supernatant samples 20x with the Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 285 µl of Dilution Buffer.

An appropriate dilution should be assessed by the researcher in advance to batch measurement. Recommended starting dilution is indicated below.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C or lower for long-term storage (not over one year). Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human IL-8.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards. Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **20 minutes** at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is less than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine IL-8 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat two times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 100**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 50**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 12.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 6.25**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 3.13**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 1.56**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of IL-8 (pg/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 2 pg/ml (from standard curve) x 6 (dilution factor) = 12 pg/ml.

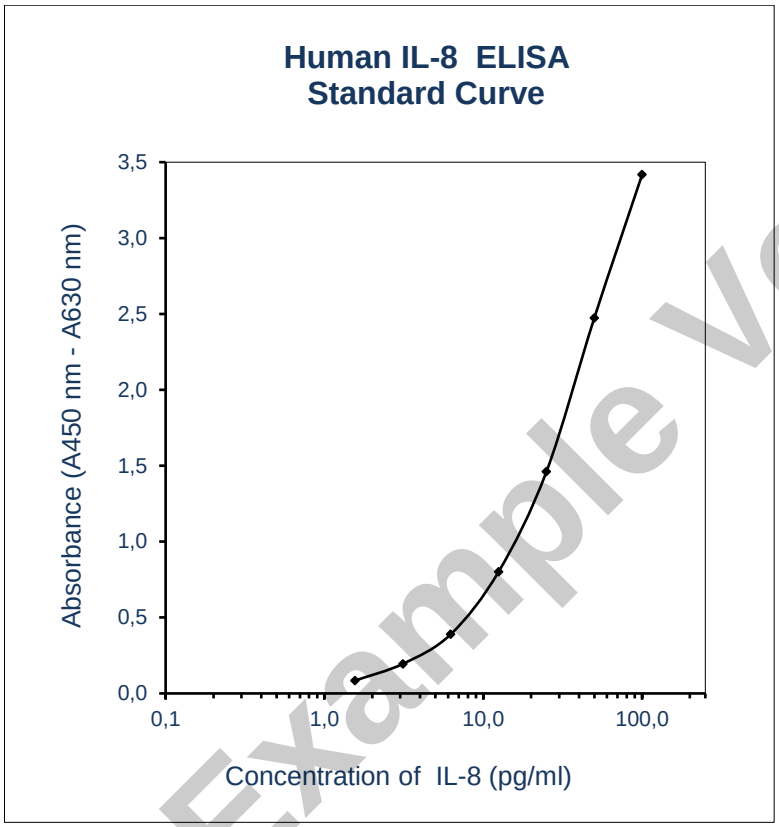


Figure 2: Typical Standard Curve for Human Interleukin-8 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human IL-8 ELISA are presented in this chapter.

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real IL-8 values in wells and is 0.5 pg/ml.
*Dilution Buffer is pipetted into blank wells.

Limit of assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

Presented results are multiplied by respective dilution factor

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	19.1	1.0	5.2
Serum 2	35.1	1.3	3.7

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	30.7	2.0	6.1
Serum 2	37.0	3.0	8.2

Spiking Recovery

Serum samples were spiked with different amounts of human IL-8 and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	41.7 61.4 75.6 108.2	- 60.3 79.5 116.7	- 101.8 95.2 92.7
Serum 2	42.9 59.2 72.0 109.3	- 61.5 80.7 117.9	- 96.2 89.2 92.7

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	- 2x 4x 8x	71.6 37.3 17.6 8.6	- 35.8 17.9 8.6	- 104.3 98.5 95.8
Serum 2	- 2x 4x 8x	68.0 34.0 17.1 8.7	- 34.0 17.0 8.5	- 100.1 100.3 102.7

Saliva samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Saliva 1	- 2x 4x 8x	1110.8 526.6 260.3 133.0	- 555.4 277.7 138.9	- 94.8 93.7 95.8
Saliva 2	- 2x 4x 8x	451.1 225.4 111.4 54.1	- 225.6 112.8 56.4	- 99.9 98.8 95.9

Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 11 individuals. Results are shown below:

Volunteer No.	Serum (pg/ml)	Plasma (pg/ml)		
		EDTA	Citrate	Heparin
1	15.0	14.9	10.0	15.7
2	5.4	4.3	5.0	2.8
3	11.0	14.0	5.7	7.2
4	2.7	3.6	2.3	2.2
5	5.1	4.0	3.4	5.0
6	2.5	2.3	2.2	2.0
7	4.0	1.5	2.2	2.3
8	5.1	1.9	2.8	3.2
9	1.9	1.8	1.5	2.7
10	8.7	5.9	5.3	6.1
11	26.2	34.6	20.0	32.9
Mean (pg/ml)	**8.0**	**8.1**	**5.5**	**7.5**
Mean Plasma/Serum (%)	**-**	**101.2**	**68.9**	**93.7**
Coefficient of determination R^2	**-**	**0.96**	**0.97**	**0.95**

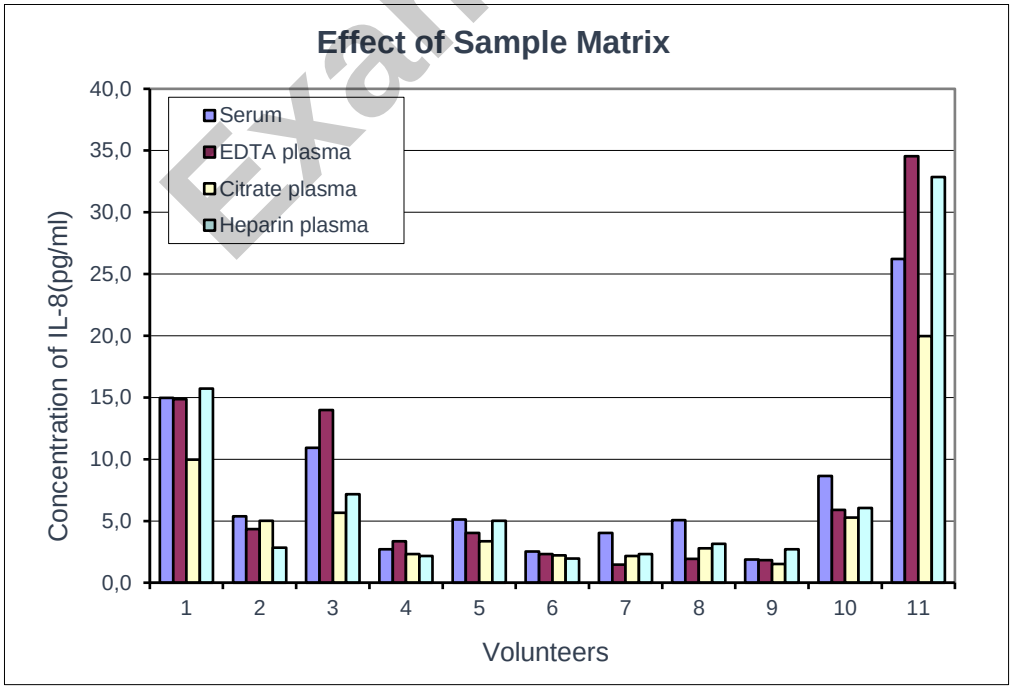


Figure 3: IL-8 levels measured using Human IL-8 ELISA using serum, EDTA, citrate and heparin plasma, respectively, from the same 11 individuals.

14. DEFINITION OF THE STANDARD

Recombinant human IL-8 is used as the standard. The recombinant IL-8 produced in *E. coli* is a 8.4 kDa protein consisting of 72 amino acid residues.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20–65 years old were assayed with the BioVendor Human IL-8 ELISA in our laboratory.

Sex	Age (years)	n	IL-8 (pg/ml)				
			Mean	Median	SD	Min	Max
Men	20-29	17	18.8	28.7	12.9	2.3	60.4
	30-39	25	19.3	14.0	15.9	5.0	84.2
	40-49	31	17.9	17.1	12.7	2.0	70.1
	50-65	16	13.4	8.9	11.3	4.1	50.7
Women	20-29	12	15.8	15.5	9.9	0.0	32.1
	30-39	26	17.3	13.6	21.0	0.0	107.9
	40-49	20	21.1	12.6	32.2	0.0	150.2
	50-61	8	13.4	9.6	8.4	5.3	29.0

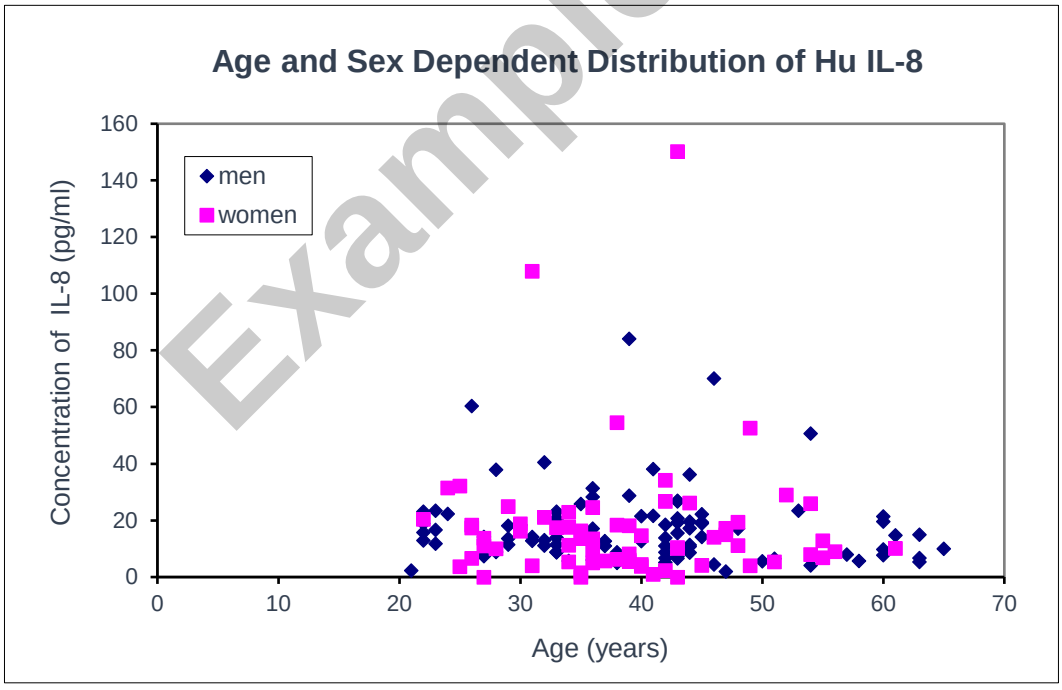


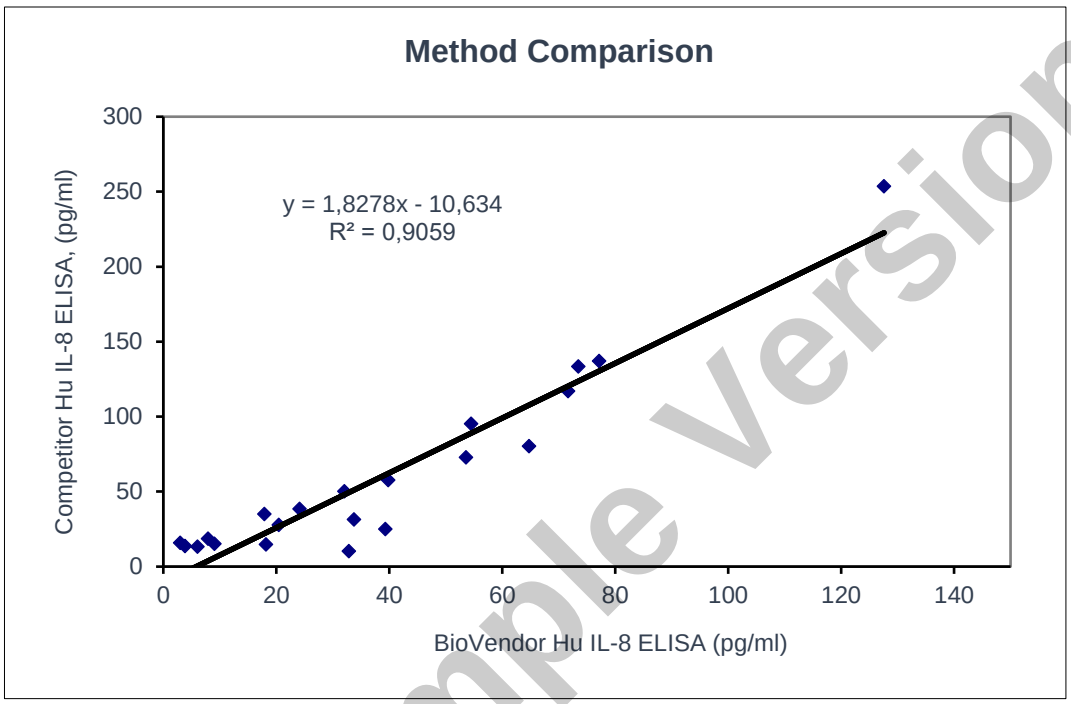
Figure 4: Human IL-8 concentration plotted against donor age and sex.

Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for IL-8 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Interleukin-8 ELISA was compared to another commercial immunoassay by measuring 21 serum samples. The following correlation graph was obtained:



17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards or samples

18. REFERENCES

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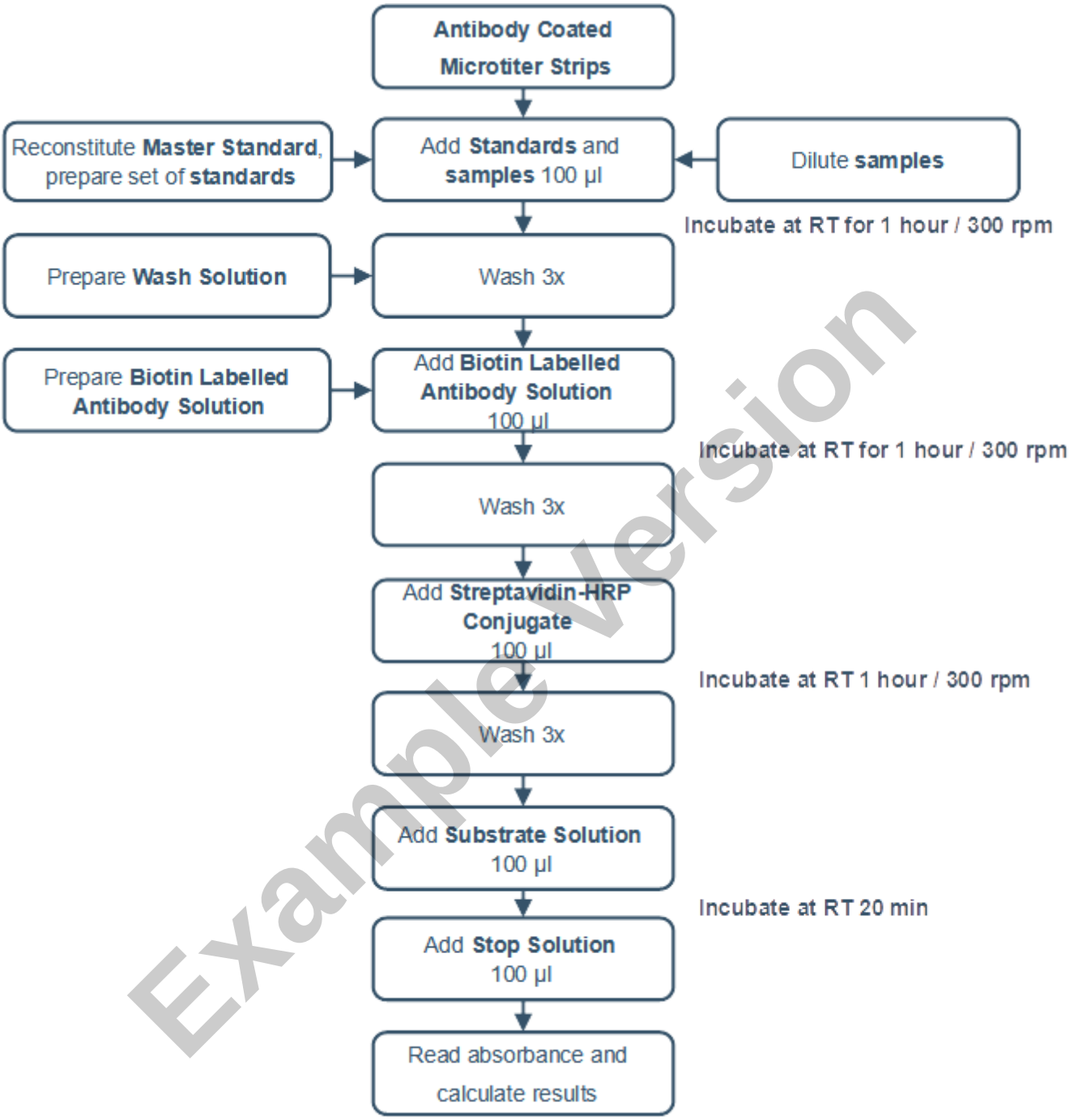
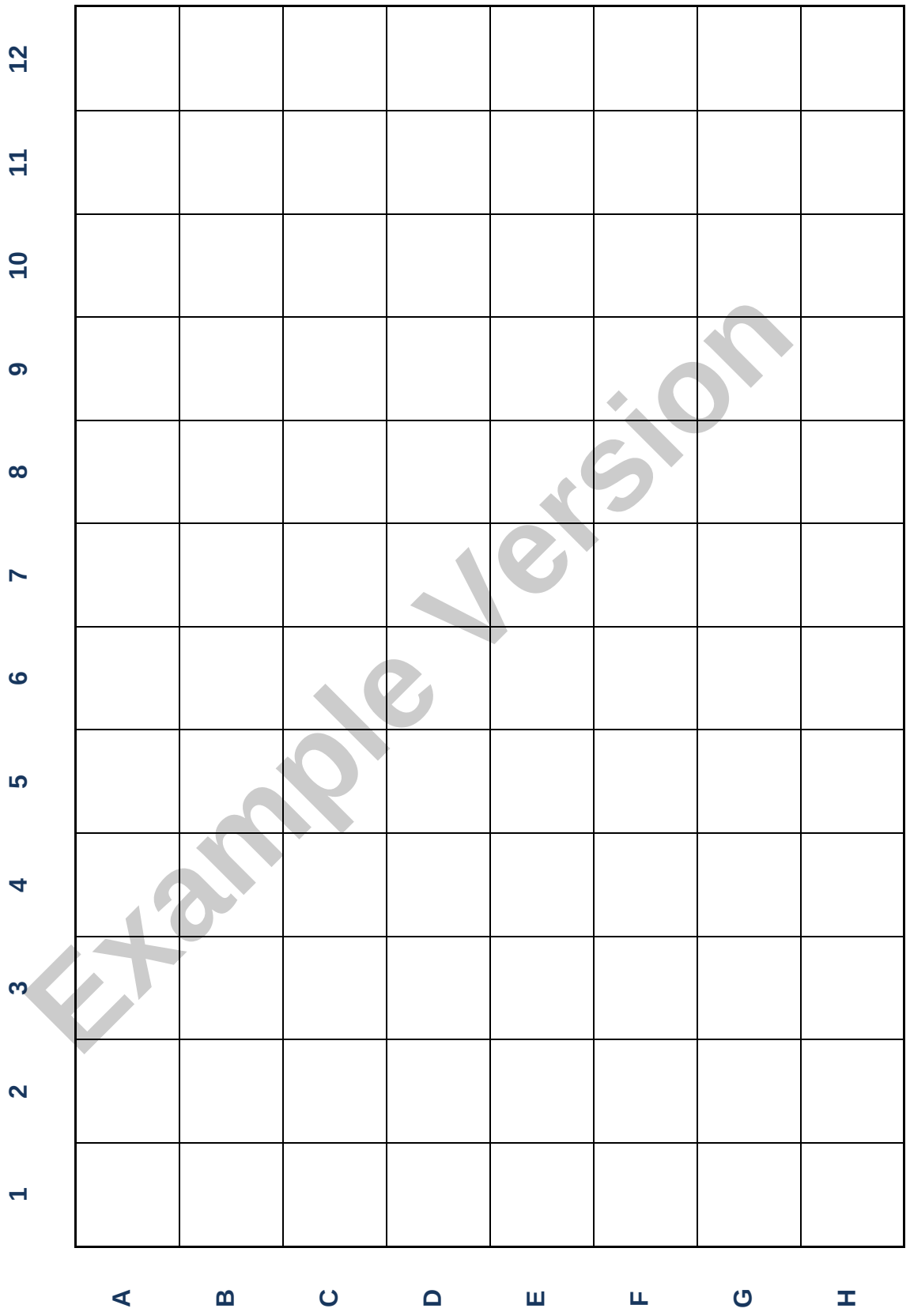
For more references on this product see our web pages at **www.biovendor.com**.

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
LOT	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

20. ASSAY PROCEDURE - SUMMARY





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