User Guide

Human IL-8 Conferma® ELISA Kit

96-Well Plate

EZHIL8-100K,
EZHIL8-100K5PK,
EZHIL8-100K10PK

Intended Use

This Human IL-8 Conferma® ELISA kit is used for the non-radioactive quantification of Human IL-8 in serum and plasma samples. One kit is sufficient to measure 38 unknown samples in duplicate. This kit is for research use only. Not for use in diagnostic procedures.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of Human IL-8 molecules from samples to the wells of a microtiter plate coated with a monoclonal mouse anti-Human IL-8 antibody
- Washing of unbound materials from samples
- Binding of a second biotinylated monoclonal mouse anti-Human IL-8 antibody to the captured molecules
- Washing of unbound materials from samples,
- Binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies
- Washing of excess free enzyme conjugates, and
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3’,5,5’-tetramethylbenzidine.

The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm–590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Human IL-8 in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Human IL-8.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Catalogue Number	Volume	Quantity
Microtiter Plate with 2 plate sealers	EP100	---------	1 plate 2 sealers
Human IL-8 Standard	E8100-K	Lyophilized	1 vial
Human IL-8 Quality Controls 1, 2 and 3	E6100-1-K E6100-2-K E6100-3-K	Lyophilized	1 vial each
Serum Matrix	EMTX-100	Lyophilized	1 vial
Assay Buffer	EAB100	10 mL	1 vial
10X Wash Buffer	EWB-HRP	50 mL	2 bottles
Human IL-8 Detection Antibody	E1100	12 mL	1 bottle
Enzyme Solution (100X)	EHRP-100	150 mL	1 bottle
Substrate Solution ESS-TMB100	ESS-TMB100	12 mL	1 bottle
Stop Solution	ET-TMB100	12 mL	1 bottle

Storage and Stability

Recommended storage for kit components is 2-8 °C.

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

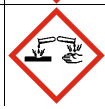
10X Wash Buffer does not contain a preservative. After dilution, the 1X Wash Buffer may be filter sterilized (Stericup® filter, Catalogue No. SCGPU11RE) for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X wash buffer should not be used after one week.

Reagent Precautions

Sodium azide has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Ingredient	Catalogue No.	Label	
Human IL-8 Standard	E8100-K		**Danger:** Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to the brain through prolonged or repeated exposure. Do not breathe dust/fume/gas/ mist/vapors/spray. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Wear protective gloves/eye protection/face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. Specific measures (see supplemental first aid instructions on this label). Rinse mouth. Remove/Take off immediately all contaminated clothing. Wash contaminated clothing before reuse. Store locked up. Dispose of contents/container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Human IL-8 Quality Control 1, 2 & 3	E6100-1-K		**Danger:** Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to the brain through prolonged or repeated exposure. Do not breathe dust/fume/gas/mist/vapors/spray. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Wear protective gloves/eye protection/face protection. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. Specific measures (see supplemental first aid instructions on this label). Rinse mouth. Remove/Take off immediately all contaminated clothing. Wash contaminated clothing before reuse. Store locked up. Dispose of contents/container to an approved waste disposal plant.
Human IL-8 Detection Antibody	E1100		**Warning:** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/fume/ gas/mist/vapors/spray. Wash skin thoroughly after handling. Wear eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Get medical advice/attention if you feel unwell. If eye irritation persists: Get medical advice/attention. Dispose of contents/container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Serum Matrix	EMTX-100		**Warning:** Causes serious eye irritation. Causes damage to organs Eyes. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Ground/bond container and receiving equipment. Wear protective gloves/protective clothing. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. IF exposed or concerned: immediately call a POISON CENTER or doctor/physician. Store in a well-ventilated place. Keep the container tightly closed.
Assay Buffer	EAB100		**Warning:** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/fume/gas/ mist/vapors/spray. Wash skin thoroughly after handling. Wear eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/container to an approved waste disposal plant.
Enzyme Solution (100X)	EHRP-100		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Enzyme Solution Diluent	ED-100		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Stop Solution	ET-TMB100		**Warning:** May be corrosive to metals.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

Sample Collection and Storage

Preparation of Serum Samples

Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 x *g*. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C.

1. Avoid multiple >2 freeze/thaw cycles.
2. Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 x *g*. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C
3. When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
4. Serum samples should be used neat.

Preparation of Plasma Samples

1. Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20 °C.
2. Avoid multiple > 2 freeze/thaw cycles.
3. When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
4. Plasma samples should be used neat.

Preparation of Tissue Culture Supernatant

1. Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20 °C.
2. Avoid multiple (> 2) freeze/thaw cycles.
3. Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

Note:

- A maximum of 50 µL per well of neat serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. DO NOT STORE SAMPLES IN GLASS.
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Reagent Preparation

Human IL-8 Standard Preparation (adjust if standard is not lyophilized)

1. Use care in opening the lyophilized Standard vial. Refer to the Standard reconstitution instructions provided on the Certificate of analysis to hydrate the stock standard vial to 1X concentration.
2. Label 5 polypropylene microfuge tubes as Std 5, Std 4, Std 3, Std 2 and Std 1.
3. Add 200 µL of Assay Buffer to each of the 5 tubes.
4. Prepare serial dilutions by adding 100 µL of the reconstituted standard to the Std 5 tube, mix well.
5. Transfer 100 µL of the Std 5 standard to the Std 4 tube, mix well.
6. Transfer 100 µL of the Std 4 standard to the Std 3 tube, mix well.
7. Transfer 100 µL of the Std 3 standard to the Std 2 tube, mix well.
8. Transfer 100 µL of the Std 2 standard to the Std 1 tube, mix well.
9. The 0 pg/mL standard (Background) will be Assay Buffer.

Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ -20 °C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard	Refer to COA	Refer to COA	200 pg/mL

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
Standard 5	200 µL	100 µL of reconstituted standard	66.7
Standard 4	200 µL	100 µL of Standard 5	22.2
Standard 3	200 µL	100 µL of Standard 4	7.4
Standard 2	200 µL	100 µL of Standard 3	2.5
Standard 1	200 µL	100 µL of Standard 2	0.8

Human IL-8 Quality Control 1, 2 and 3 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human IL-8 Quality Control 1, 2, and 3 as per the instructions provided in the Certificate of Analysis. Once hydrated, controls can be stored in small aliquots at ≤ -20 °C. Avoid further freeze/thaw cycles.

Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 100 mL of 10X Wash Buffer (two bottles) with 900 mL deionized water. Store unused portion at 2-8 °C for up to one month.

Preparation of Serum Matrix

Add 1.5 mL distilled or de-ionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 15 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20 °C for up to one month.

Preparation of Enzyme Solution

Add 120 µL of 100X enzyme solution to the bottle containing 12 mL of enzyme solution diluent. Mix well. Store unused portion at 2-8 °C for up to one month.

Human IL-8 ELISA Assay Procedure

Warm all reagents to room temperature before setting up the assay.

1. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder. Add 300 µL diluted Wash Buffer to each well of the plate. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.
2. Add 50 µL of appropriate Matrix Solution to Blank, Standards and Quality Control wells (refer to Microtiter Plate Arrangement section for suggested sample order placement). When assaying serum or plasma, use EMTX-100. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
3. Add 50 µL Assay Buffer to each of the Blank and Sample wells.
4. Add 50 µL Standards or Controls to the appropriate wells.
5. Add 50 µL of sample to the appropriate wells.
6. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.
7. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
8. Add 100 µL Detection Antibody to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.
9. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
10. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.
11. Remove sealer, decant reagents from the plate and tap plate to remove the residual volume. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

12. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 15-20 minutes. Blue color should be formed in wells of the Human IL-8 standards with intensity proportional to increasing concentrations of Human IL-8.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

13. Remove sealer and add 100 µL Stop Solution (CAUTION: CORROSIVE SOLUTION) and gently shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest Human IL-8 standard should be approximately 2.0-3.0, or not to exceed the capability of the plate reader used.

    Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example, if 25 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 µL, compensate for the volume deficit with Assay Buffer.

Assay Procedure for Human IL-8 ELISA Kit

	Step 1	Step 2	Step 3	Step 4-5	Step 6-7	Step 8	Step 9	Step 10	Step 11	Step 12-13		
Well #		Matrix Solution	Assay Buffer	Standards/ QCs/Samples		Detection Antibody		Enzyme Solution		Substrate		Stop
A1, B1	Wash plate 1X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	50 µL	50 µL	--	Seal, Agitate, Incubate 2 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 1 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate 30 minutes at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	100 µL	Seal, Agitate, Incubate for 15-20 minutes at Room Temperature.	100 µL	Read Absorbance at 450 nm and 590 nm.
C1, D1		50 µL	--	50 µL of Std 1								
E1, F1		50 µL	--	50 µL of Std 2								
G1, H1		50 µL	--	50 µL of Std 3								
A2, B2		50 µL	--	50 µL of Std 4								
C2, D2		50 µL	--	50 µL of Std 5								
E2, F2		50 µL	--	50 µL of Reconstituted standard								
G2, H2		50 µL	--	50 µL of QC 1								
A3, B3		50 µL	--	50 µL of QC 2								
C3, D3		50 µL	50 µL	50 µL of QC 3								
E3, F3		--	50 µL	50 µL of sample								
G3, H3, etc.		--	50 µL	50 µL of sample								

Microtiter Plate Arrangement

Human IL-8 ELISA

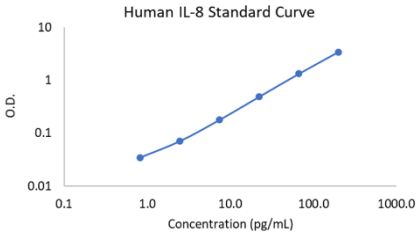
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std 4	QC 2									
B	Blank	Std 4	QC 2									
C	Std 1	Std 5	QC 3									
D	Std 1	Std 5	QC 3									
E	Std 2	Reconstituted Standard	Sample [#]									
F	Std 2	Reconstituted Standard	Sample [#]									
G	Std 1	QC 1										
H	Std 3	QC 1										

Assay Characteristics

Sensitivity

The Minimum Detectable Concentration (MinDC) of Human IL-8 is 0.8 pg/mL. It is calculated by using Belysa® Immunoassay Curve Fitting Software (40-122). It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. This reported value is the mean plus 2 standard deviations of the MinDC of multiple assays (n= # assays).

Graph of Typical Reference Curve



Typical Standard Curve, not to be used to calculate data.

Specificity

The antibody pair used in this assay is specific to Human IL-8 and does not significantly cross-react to the following molecules/hormones tested: Human IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, MCP-3, MIP1α, MIP1β, GROα, RANTES, MCP-1

Precision

Mean Intra-assay precision is calculated from the results of twenty replicates each of the three different concentrations of human IL-8 in a single assay. The mean inter-assay precision is generated from the results of eight separate assays with duplicate samples in each assay for the three different concentrations of IL-8.

Intra-Assay Variation

	Mean Human IL-8 Levels (pg/mL)	Intra-Assay %CV
1	4.7	4.0
2	14.6	3.4
3	42.7	3.5

Inter-Assay Variation

	Mean Human IL-8 Levels (pg/mL)	Inter-Assay %CV
1	4.4	4.7
2	13.3	5.4
3	41.6	3.7

The assay variations of our Human IL-8 ELISA kit was studied on two samples at two levels on the Human IL-8 standard curve. The mean intra-assay variation was calculated from results of eight determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of 3 separate assays with duplicate samples in each assay.

Spike Recovery of Human IL-8 in Assay Samples

Sample	Spiked Concentration of IL-8 (pg/mL)	Expected in the assay (pg/mL)	Recovery
Serum 1	0	0.4	
	2.47	2.8	97
	7.41	7.5	96
	22.2	21.8	96
Serum 2	0	2.6	
	2.47	5.1	101
	7.41	9.3	91
	22.2	24.6	99
Serum 3	0	1.0	
	2.47	3.4	95
	7.41	8.6	102
	22.2	24.3	105
Serum 4	0	3.3	
	2.47	5.8	100
	7.41	8.6	70
	22.2	22.7	87
Serum 5	0	3.9	
	2.47	6.3	97
	7.41	10.1	84
	22.2	23.9	90
Average			94

Sample	Spiked Concentration of IL-8 (pg/mL)	Expected in the assay (pg/mL)	Recovery
Plasma 1	0	0.0	
	2.47	2.0	80
	7.41	6.1	83
	22.2	19.2	87
Plasma 2	0	0.2	
	2.47	2.2	80
	7.41	6.3	82
	22.2	19.0	84
Plasma 3	0	1.2	
	2.47	3.2	81
	7.41	7.1	80
	22.2	20.3	86
Plasma 4	0	1.0	
	2.47	3.2	89
	7.41	7.7	90
	22.2	21.3	91
Plasma 5	0	1.0	
	2.47	3.5	102
	7.41	6.7	78
	22.2	18.2	77
Average			85

Varying amounts of Human IL-8 were added to individual human serum and plasma samples and the resulting Human IL-8 content of each sample was assayed by Human IL-8 ELISA. The recovery = [(observed Human IL-8 / spiked Human IL-8 concentration) + basal Human IL-8 level] x 100%.

Linearity of Sample Dilution

Sample	Neat Sample volume in 50 µL total volume (µL)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity%
Serum 1	50	14.3	14.3	
	25	7.6	15.3	107
	12.5	3.9	15.7	110
	6.25	2.1	16.6	116
Serum 2	50	17.1	17.1	
	25	8.9	17.9	104
	12.5	4.4	17.5	102
	6.25	2.3	18.1	106
Serum 3	50	15.5	15.5	
	25	8.1	16.1	104
	12.5	3.9	15.7	101
	6.25	2.0	16.2	104
Serum 4	50	32.6	32.6	
	25	17.0	34.0	105
	12.5	8.5	33.9	104
	6.25	4.5	35.6	109
Serum 5	50	184.8	184.8	
	25	94.7	189.4	102
	12.5	44.9	179.4	97
	6.25	23.8	190.0	103
Average				105

Sample	Neat Sample volume in 50 µL total volume (µL)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity%
Plasma 1	50	14.0	14.0	
	25	7.1	14.2	101
	12.5	3.6	14.6	104
	6.25	2.0	15.6	111
Plasma 2	50	13.0	13.0	
	25	6.5	13.0	100
	12.5	3.5	13.8	106
	6.25	1.9	14.9	115
Plasma 3	50	12.9	12.9	
	25	6.6	13.2	102
	12.5	3.3	13.3	103
	6.25	1.8	14.6	113
Plasma 4	50	17.2	17.2	
	25	8.5	17.1	99
	12.5	4.3	17.3	100
	6.25	2.3	18.1	105
Plasma 5	50	15.7	15.7	
	25	8.1	16.1	103
	12.5	4.1	16.3	104
	6.25	2.1	16.9	108
Average				105

Ten spiked individual human serum and plasma samples with the indicated sample volumes were assayed. Required amounts of Assay Buffer were added to compensate for lost volumes below 50 µL. Neat sample volumes of 50 µL, 25 µL, 12.5 µL, and 6.25 µL in a 50 µL total sample volume represents dilution factors of 1, 2, 4, and 8, respectively, were applied in the calculation of observed Human IL-8 concentrations.

$$\% \text{ expected} = (\text{observed}/\text{expected}) \times 100\%$$

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High signal in background or blank wells could be due to:
    - cross well contamination by standard solution or sample, or
    - inadequate washing of wells with Wash Buffer, or
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Catalogue Number
Human IL-8 ELISA plate with 2 sealers	EP100
10X HRP Wash Buffer Concentrate	EWB-HRP
Human IL-8 ELISA Standard	E8100-K
Human IL-8 Quality Controls 1, 2 and 3	E6100-1-K E6100-2-K E6100-3-K
Serum Matrix	EMTX-100
Assay Buffer	EAB100
Human IL-8 ELISA Detection Antibody	E1100
Enzyme Solution (100X)	EHRP-100
Enzyme Solution Diluent	ED-100
Substrate Solution	ESS-TMB100
Stop Solution	ET-TMB100

Notice

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