

## User Guide

**Human IL-6 Conferma® ELISA Kit**

## 96-Well Plate

**EZIL6-98K,  
(EZIL6-98K5PK,  
EZIL6-98K10PK)**

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## Intended Use

This Human IL-6 ELISA kit is used for the quantification of Human IL-6 in serum and plasma samples. One kit is sufficient to measure 36 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-6 molecules from samples to the wells of a microtiter plate coated with a monoclonal mouse anti-Human IL-6 antibody
- Washing of unbound materials from samples
- Binding of a second biotinylated monoclonal mouse anti-Human IL-6 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-6 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-6.

## 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
Human IL-6 ELISA plate with 2 sealers	EP98	-----	1 plate 2 sealers
Human IL-6 Standard	E8098-K	Lyophilized	1 vial
Human IL-6 Quality Controls 1, 2 and 3	E6098-1-K E6098-2-K E6098-3-K	Lyophilized	1 vial each
Serum Matrix	EMTX-98	Lyophilized	1 vial
Assay Buffer	EAB098	10 mL	1 vial
10X Wash Buffer	EWB-HRP98	50 mL	2 bottles
Human IL-6 Detection Antibody	E1098	12 mL	1 bottle
Enzyme Solution (100X)	EHRP-98	150 µL	1 bottle
Enzyme Solution Diluent	ED-098	12 mL	1 bottle
Substrate Solution	ESS-TMB98	12 mL	1 bottle
Stop Solution	ET-TMB98	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.







Refer to expiration dates on all reagents before use. Do not mix reagents from different kits unless they have the same lot numbers.

## 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-6 Standard	E8098-K	  	<b>Danger:</b> 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.

For research use only. Not for use in diagnostic procedures.

Ingredient	Catalogue No.	Label	
Human IL-6 Quality Control 1, 2 & 3	E6098-1-K E6098-2-K E6098-3-K	  	<p><b>Danger:</b> 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.</p>
Human IL-6 Detection Antibody	E1098		<p><b>Warning:</b> Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing.</p>
Serum Matrix	EMTX-98	No label	<p>Harmful to aquatic life with long lasting effects. Avoid release to the environment.</p>
Assay Buffer	EAB098		<p><b>Warning:</b> Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing.</p>
Stop Solution	ET-TMB98		<p><b>Warning:</b> May be corrosive to metals.</p>

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Ingredient	Catalogue No.	Label	
Substrate	ESS-TMB98	   	<p><b>Danger:</b> Highly flammable liquid and vapour. Toxic if swallowed, in contact with skin, or if inhaled. Causes serious eye irritation. Causes damage to Eyes. Keep away from heat/sparks/open flames/hot surfaces. No smoking.</p> <p>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 container tightly closed.</p>

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## Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5  $\mu\text{L}$ -50  $\mu\text{L}$  and 50  $\mu\text{L}$ -300  $\mu\text{L}$
- Pipettes and pipette tips: 10  $\mu\text{L}$ -20  $\mu\text{L}$  or 20  $\mu\text{L}$ -100  $\mu\text{L}$
- 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  $\times g$ . Remove serum and assay immediately or aliquot and store samples at  $\leq -20$   $^{\circ}\text{C}$ .

1. Avoid multiple  $> 2$  freeze/thaw cycles.
2. 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.
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  $\leq -20$  °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. 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.
5. 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  $\leq -20$  °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  $\mu$ L 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.

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## Reagent Preparation

Human IL-6 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 7 polypropylene microfuge tubes as Std 7, Std 6, Std 5, Std 4, Std 3, Std 2, and Std 1.
3. Add 200  $\mu$ L of Assay Buffer to each of the 7 tubes.
4. Prepare serial dilutions by adding 200  $\mu$ L of the reconstituted standard to the Std 7 tube, mix well.
5. Transfer 200  $\mu$ L of the Std 7 standard to the Std 6 tube, mix well.
6. Transfer 200  $\mu$ L of the Std 6 standard to the Std 5 tube, mix well.
7. Transfer 200  $\mu$ L of the Std 5 standard to the Std 4 tube, mix well.
8. Transfer 200  $\mu$ L of the Std 4 standard to the Std 3 tube, mix well.
9. Transfer 200  $\mu$ L of the Std 3 standard to the Std 2 tube, mix well.
10. Transfer 200  $\mu$ L of the Std 2 standard to the Std 1 tube, mix well. 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  $\leq -20$  °C. Avoid multiple freeze/thaw cycles.

<b>Tube #</b>	<b>Volume of Deionized Water to Add</b>	<b>Volume of Standard to Add</b>	<b>Standard Stock Concentration</b>
Reconstituted standard	Refer to COA	Refer to COA	150 pg/mL
<b>Tube #</b>	<b>Volume of Assay Buffer to Add</b>	<b>Volume of Standard to Add</b>	<b>Standard Concentration (pg/mL)</b>
Standard 7	200 µL	200 µL of reconstituted standard	75
Standard 6	200 µL	200 µL of Standard 7	37.5
Standard 5	200 µL	200 µL of Standard 6	18.75
Standard 4	200 µL	200 µL of Standard 5	9.38
Standard 3	200 µL	200 µL of Standard 4	4.69
Standard 2	200 µL	200 µL of Standard 3	2.34
Standard 1	200 µL	200 µL of Standard 2	1.17

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## Human IL-6 Quality Control 1, 2 and 3 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human IL-6 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  $\leq -20$  °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  $\leq -20$  °C for up to one month.

### Preparation of Enzyme Solution

Add 120  $\mu$ L 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-6 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. **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 Catalogue No. EMTX-98. 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 5 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 minutes. Blue color should be formed in wells of the Human IL-6 standards with intensity proportional to increasing concentrations of Human IL-6.

**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.

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13. Remove sealer and add 100  $\mu$ L 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-6 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  $\mu$ L, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example, if 25  $\mu$ L of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50  $\mu$ L, compensate for the volume deficit with Matrix Solution or buffer.

## Assay Procedure for Human IL-6 ELISA Kit

Well #	Step 1	Step 2	Step 3	Step 4-5	Step 6-7	Step 8	Step 9	Step 10	Step 11	Step 12-13
A1, B1	Wash plate 1X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	Matrix Solution			Seal, Agitate, Incubate 2 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	Detection Antibody				
C1, D1		50 µL	--	50 µL of Std2						
E1, F1		50 µL	--	50 µL of Std3						
G1, H1		50 µL	--	50 µL of Std4						
A2, B2		50 µL	--	50 µL of Std5						
C2, D2		50 µL	--	50 µL of Std6						
E2, F2		50 µL	--	50 µL of Std7						
G2, H2		50 µL	--	50 µL of Reconstituted standard						
A3, B3		50 µL		50 µL of QC1						
C3, D3		50 µL		50 µL of QC2						
E3, F3		50 µL		50 µL of QC3						
G3, H3, etc.		--	50 µL	50 µL of sample						
						100 µL				
								100 µL		
									100 µL	
										Substrate
										Seal, Agitate, Incubate for 15 minutes at Room Temperature.
										Stop
										Read Absorbance at 450 nm and 590 nm.

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# Microtiter Plate Arrangement

## Human IL-6 ELISA

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

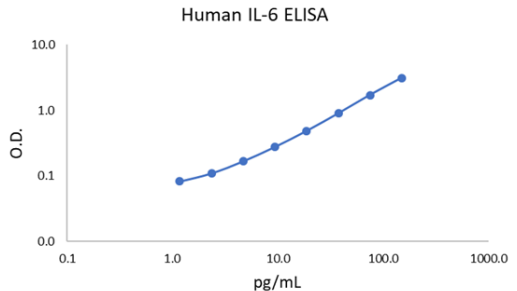
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## Assay Characteristics

### Sensitivity

The Minimum Detectable Concentration (MinDC) of IL-6 is 1.17 pg/mL. It is calculated by using [Belysa<sup>®</sup> 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.

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 IL-6 and does not significantly cross-react to the following molecules/hormones tested:

Mouse IL-6, Human IL-1a, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, TNF $\alpha$ , TNF $\beta$ , GMCSF, GCSF

## Precision

### Intra-Assay Variation

	<b>Mean IL-6 Levels (pg/mL)</b>	<b>Intra-Assay %CV</b>
1	3.6	4.1
2	10.6	2.1
3	32.1	1.6

### Inter-Assay Variation

	<b>Mean IL-6 Levels (pg/mL)</b>	<b>Intra-Assay %CV</b>
1	3.1	12.9
2	9.6	7.4
3	28.3	6.5

The assay variations of our IL-6 ELISA kit was studied on two samples at two levels on the IL-6 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 8 separate assays with duplicate samples in each assay.

## Spike Recovery of IL-6 in Assay Samples

Sample	Spiked Concentration of IL-6 Added (pg/mL)	Observed (pg/mL)	Recovery
1	0	0	
	4.7	4.4	94
	9.4	9.0	96
	18.8	17.7	95
2	0	0.0	
	4.7	5.0	107
	9.4	9.9	105
	18.8	18.9	101
3	0	3.5	
	4.7	8.2	99
	9.4	12.6	97
	18.8	22.1	99
4	0	0.4	
	4.7	4.8	93
	9.4	9.2	94
	18.8	18.0	94
5	0	0.3	
	4.7	4.6	91
	9.4	8.9	91
	18.8	17.5	91
Average			93

Varying amounts of Human IL-6 were added to individual human serum and plasma samples and the resulting IL-6 content of each sample was assayed by IL-6 ELISA.

The recovery =

$[(\text{observed Basal} / \text{spiked IL-6 concentration}) + \text{basal IL-6 level}] \times 100\%$ .

<b>Sample</b>	<b>Spiked Concentration of IL-6 Added (pg/mL)</b>	<b>Observed (pg/mL)</b>	<b>Recovery</b>
Plasma 1	0	0.63	
	4.7	5.6	105
	9.4	10.0	100
	18.8	21.2	109
Plasma 2	0	0.0	
	4.7	4.5	96
	9.4	9.3	99
	18.8	19.7	105
Plasma 3	0	0.7	
	4.7	5.6	104
	9.4	10.1	99
	18.8	20.5	105
Plasma 4	0	0.4	
	4.7	5.3	104
	9.4	9.9	101
	18.8	19.2	100
Plasma 5	0	0.4	
	4.7	5.2	104
	9.4	10.1	104
	18.8	20.8	109
<b>Average</b>			<b>103</b>

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## Linearity of Sample Dilution

<b>Sample</b>	<b>Volume (μL)</b>	<b>Mean (pg/mL)</b>	<b>Dilution Corrected (pg/mL)</b>	<b>Linearity</b>
Serum 1	50	16.1	16.1	
	25	8.5	17.0	106
	12.5	4.4	17.8	111
	6.25	2.3	18.6	116
Serum 2	50	17.7	17.7	
	25	9.5	19.0	108
	12.5	4.9	19.6	111
	6.25	2.5	20.2	114
Serum 3	50	20.3	20.3	
	25	10.7	21.5	106
	12.5	5.7	22.7	112
	6.25	2.8	22.6	112
Serum 4	50	18.0	18.0	
	25	9.5	18.9	105
	12.5	4.9	19.7	110
	6.25	2.5	20.3	113
Serum 5	50	16.7	16.7	
	25	9.0	18.0	108
	12.5	4.7	18.8	112
	6.25	2.4	19.2	115
<b>Average</b>				<b>110</b>

Sample	Volume (µL)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity
Plasma 1	50	20.0	20.0	
	25	10.0	19.9	100
	12.5	4.9	19.6	98
	6.25	2.2	17.7	88
Plasma 2	50	19.6	19.6	
	25	9.5	19.0	97
	12.5	4.5	18.2	93
	6.25	2.3	18.3	93
Plasma 3	50	19.8	19.8	
	25	9.8	19.7	100
	12.5	5.0	19.9	101
	6.25	2.3	18.7	95
Plasma 4	50	21.0	21.0	
	25	10.3	20.5	98
	12.5	5.2	20.7	98
	6.25	2.5	19.9	94
Plasma 5	50	19.7	19.7	
	25	9.8	19.6	100
	12.5	5.0	20.0	102
	6.25	2.6	20.6	105
Average				97

**Note:** More data related to assay characteristics can be found in Human IL-6 Conferma® ELISA verification report.

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Ten spiked individual human serum and plasma samples with the indicated sample volumes were assayed. Required amounts of serum matrix were added to compensate for lost volumes below 50  $\mu\text{L}$ . The resulting dilution factors of neat, 2, 4 and 8 representing 50  $\mu\text{L}$ , 25  $\mu\text{L}$ , 12.5  $\mu\text{L}$  and 6.25  $\mu\text{L}$  sample volumes assayed, respectively, were applied in the calculation of observed IL-6 concentrations.

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

## Quality Controls

The ranges for each analyte in Quality Control 1, 2 and 3 are provided on the card insert, or available at our website [SigmaAldrich.com](http://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](https://www.sigmaaldrich.com).

### Replacement Reagents

<b>Reagents</b>	<b>Catalogue Number</b>
Human IL-6 ELISA plate with 2 sealers	EP98
Human IL-6 Standard	E8098-K
Human IL-6 Quality Controls 1, 2 and 3	E6098-1-K
	E6098-2-K
	E6098-3-K
Serum Matrix	EMTX-98
Assay Buffer	EAB098
10X Wash Buffer	EWB-HRP98
Human IL-6 Detection Antibody	E1098
Enzyme Solution (100X)	EHRP-98
Enzyme Solution Diluent	ED-098
Substrate Solution	ESS-TMB98

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