# Human IL-6 Conferma™ ELISA

## 96-Well Plate Assay

## Cat. # EZIL6-98K (EZIL6-98K5PK, EZIL6-98K10PK)

# HUMAN IL-6 CONFERMA™ ELISA KIT
## 96-Well Plate

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## # (EZIL6-98K5PK, EZIL6-98K10PK)

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## INTENDED USE

This Human IL-6 Conferma™ ELISA kit is used for the non-radioactive 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 which uses in-house developed critical reagents, including the Monoclonal Antibodies (mAb) and calibration material, to detect endogenous IL-6 in biological fluids such as Human serum or plasma.

The Sandwich ELISA first binds IL-6 using a specific capture Mouse anti-human IL-6 monoclonal antibody bound to the wells of a 96 well microtitre plate. Following the addition of the sample, the assay is incubated for two hours, during which time endogenous or recombinant antigen (depending on the well) is bound by the mAb. The unbound material is washed off post-incubation, and a biotinylated mouse anti-human IL-6 monoclonal antibody is added to complete the “Sandwich”. After an incubation period, the unbound material is washed off. The next step is a final incubation step during which a streptavidin-horseradish peroxidase conjugate binds to the immobilized biotinylated antibodies, and following a final wash horseradish peroxidase substrate, 3,3’,5,5’-tetramethylbenzidine is added. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450-590 nm after acidification of formed products by the addition of Stop Solution. The increase in absorbance is directly proportional to the amount of captured Human IL-6. Quantitation of the analyte is derived by interpolation from a reference curve comprised of standard points of known concentrations of recombinant 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 | Catalog Number | Volume | Quantity |
|---|---|---|---|
| Human IL-6 ELISA plate with 2 sealers | EP98 | --------- | 1 plate<br>2 sealers |
| Human IL-6 Standard | E8098-K | lyophilized | 1 vial |
| Human IL-6 Quality Controls 1, 2 and 3 | E6098-1-K<br>E6098-2-K<br>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

The 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.

10X Wash Buffer does not contain a preservative. After dilution, the 1X Wash Buffer may be filter sterilized (Stericup® filter, Millipore Sigma- Cat# 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 concentration is 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.

## FULL HAZARD LABEL:

| Ingredient, Cat # | | Full Label | |
|---|---|---|---|
| Human IL-6 Standard | E8098-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-6 Quality Control 1, 2 & 3 | E6098-1-K<br>E6098-2-K<br>E6098-3-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-6 detection antibody | E1098 |  | **Warning:** 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. |
| Serum Matrix | EMTX-98 | No label | Harmful to aquatic life with long lasting effects. Avoid release to the environment. |
| Assay Buffer | EAB098 |  | **Warning:** 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. |
| Stop Solution | ET-TMB98 |  | **Warning:** May be corrosive to metals. |

## MATERIALS REQUIRED BUT NOT PROVIDED

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

## SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >2 freeze/thaw cycles.
- 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.
- Serum samples should be used neat.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anticoagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >2 freeze/thaw cycles.
- 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.
- Plasma samples should be used neat.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require 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 anticoagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

## REAGENT PREPARATION

### A. Human IL-6 Standard Preparation

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. For dilution series, Label 7 polypropylene microfuge tubes as Std 7, Std 6, Std 5, Std 4, Std 3, Std 2, and Std 1. Add 200 µL of Assay Buffer to each of the 7 tubes. Prepare serial dilutions by adding 200 µL of the reconstituted standard to the Std 7 tube, mix well and transfer 200 µL of the Std 7 to the Std 6 tube, mix well and transfer 200 µL of the Std 6 to the Std 5 tube, mix well and transfer 200 µL of the Std 5 to the Std 4 tube, mix well and transfer 200 µL of the Std 4 to the Std 3 tube, mix well and transfer 200 µL of the Std 3 to the Std 2 tube, mix well and transfer 200 µL of the Std 2 to the Std 1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.
   Note: Change tip for every dilution. Wet tip with the standard before dispensing. Unused portions of the 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 Assay Buffer to Add | Standard Stock Concentration |
|---|---|---|---|
| Reconstituted standard | Refer to COA | Refer to COA | 150 pg/mL |

| Tube # | Volume of Assay Buffer to Add | Volume of Standard to Add | Standard Concentration pg/mL |
|---|---|---|---|
| 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 |

### B. 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 ≤ -20°C. Avoid further freeze/thaw cycles.

## REAGENT PREPARATION (continued)

### C. 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 of de-ionized water.

**NOTE:** 10X Wash Buffer does not contain a preservative. For storage of up to 1 month at 2 - 8°C, the 1X Wash Buffer may need to be filter sterilized (Stericup® filter, Millipore Sigma- Cat# SCGPU11RE)

### D. 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.

### E. 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-6 Conferma™ 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-98. When assaying tissue culture or other supernatants, 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 neat sample to the appropriate wells.

6. Cover the plate with a 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.

## Human IL-6 Conferma™ ELISA ASSAY PROCEDURE (continued)

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 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 of 1X Enzyme Solution to each well. Cover the 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 the 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 IL-6 standards with intensity proportional to increasing concentrations of 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.**

13. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and gently shake plate by hand to ensure complete mixing of the 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 a 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 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 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 25 µL of sample is used, then calculated data must be multiplied by 2). When the sample volume assayed is less than 50 µL, compensate for the volume deficit with the matrix solution.

## Assay Procedure for Human IL-6 Conferma™ ELISA Kit (Cat. # EZIL6-98K)

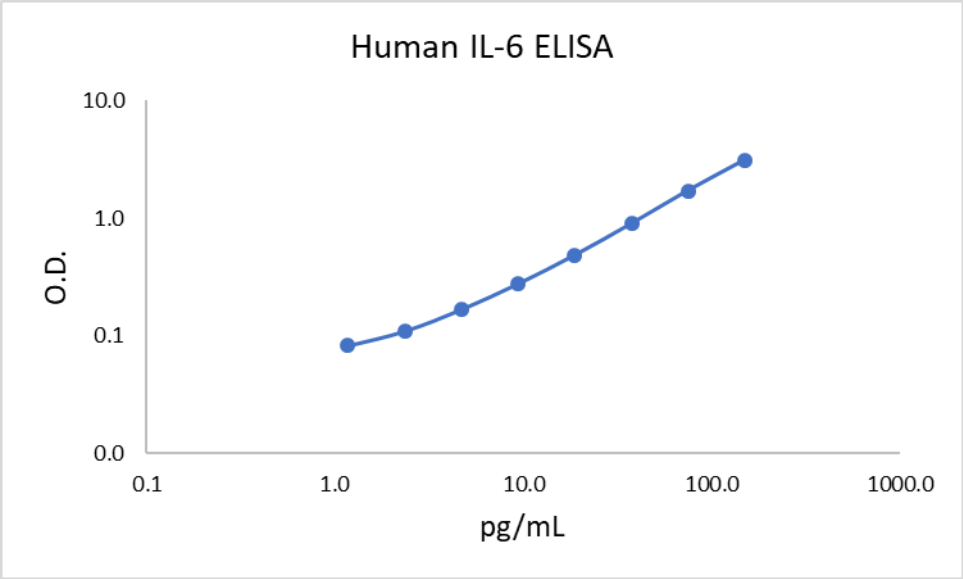
| | Step 1 | Step 2 | Step 3 | Step 4-5 | Step 6-7 | Step 8 | Step 9 | Step 10 | Step 11 | Step 12-13 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Well # | **Wash plate 1X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.** | Matrix Solution | Assay Buffer | Standards/ QCs/ Samples | **Seal, Agitate, Incubate 2 hour at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.** | Detection Antibody | **Seal, Agitate, Incubate 1 hour at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.** | Enzyme Solution | **Seal, Agitate, Incubate 30 minutes at Room Temperature on a plate shaker. Wash 5X with 300 µL Wash Buffer.** | Substrate | **Seal, Agitate, Incubate 15 minutes at Room Temperature on a plate shaker.** | Stop | **Read Absorbance at 450 nm and 590 nm.** |
| A1, B1 | | 50 µL | **50** µL | -- | | 100 µL | | 100 µL | | 100 µL | | 100 µL | |
| C1, D1 | | 50 µL | -- | 50 µL of Std1 | | | | | | | | | |
| 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 Std 6 | | | | | | | | | |
| G2, H2 | | 50 µL | -- | 50 µL of Std 7 | | | | | | | | | |
| A3, B3 | | 50 µL | -- | 50 µL of Reconstituted standard | | | | | | | | | |
| C3, D3 | | 50 µL | | 50 µL of QC 1 | | | | | | | | | |
| E3, F3 | | 50 µL | | 50 µL of QC 2 | | | | | | | | | |
| G3, H3. | | 50 µL | | 50 µL of QC 3 | | | | | | | | | |
| A4, B4 Etc. | | | **50** µL | 50 µL of sample | | | | | | | | | |

## MICROTITER PLATE ARRANGEMENT

Human IL-6 Conferma™ 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 | QC1 | | | | | | | | | |
| D | Std 1 | Std 5 | QC1 | | | | | | | | | |
| E | Std 2 | Std 6 | QC2 | | | | | | | | | |
| F | Std 2 | Std 6 | QC2 | | | | | | | | | |
| G | Std 3 | Std 7 | QC3 | | | | | | | | | |
| H | Std 3 | Std 7 | QC3 | | | | | | | | | |

## GRAPH OF TYPICAL REFERENCE CURVE




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

## ASSAY CHARACTERISTICS

### A. Sensitivity

The lower limit of quantitation (LLOQ) of IL-6 assay is 1.17 pg/mL using Belysa™ Immunoassay Analysis software from Millipore Sigma. LLOQ is calculated by back interpolation of the standard point that provides CV≤ 20% and recovery ± 20% of the expected.

### B. Specificity

The antibody pair used in this assay is specific to Human IL-6 and does not 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, TNFα, TNFβ, GMCSF, GCSF

### C. Precision

Mean Intra-assay precision is calculated from the results of twenty replicates each of the three different concentrations of human IL-6 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-6.

## ASSAY CHARACTERISTICS (continued)

Intra-Assay Variation

| | Mean IL-6 Levels pg/mL | Intra-Assay %CV |
|---|---|---|
| 1 | 3.6 | 4.1 |
| 2 | 10.6 | 2.1 |
| 3 | 32.1 | 1.6 |

Inter-Assay Variation

| | Mean IL-6 Levels pg/mL | Inter-Assay %CV |
|---|---|---|
| 1 | 3.1 | 12.9 |
| 2 | 9.6 | 7.4 |
| 3 | 28.3 | 6.5 |

### D. Spike Recovery of IL-6 in Blood Samples

Varying amounts of Human IL-6 were added to 10 individual human serum and plasma samples, and the resulting IL-6 content of each sample was assayed by Human IL-6 Conferma™ ELISA.
The recovery = [(observed- Basal / (spiked IL-6 concentration)] x 100%.

| Sample | Spiked Concentration of IL-6 pg/mL | Concentration observed in the assay (pg/mL) | Recovery% |
|---|---|---|---|
| Serum 1 | 0 | 0 | |
| | 4.7 | 4.4 | 94 |
| | 9.4 | 9.0 | 96 |
| | 18.8 | 17.7 | 95 |
| | | | |
| Serum 2 | 0 | 0.0 | |
| | 4.7 | 5.0 | 107 |
| | 9.4 | 9.9 | 105 |
| | 18.8 | 18.9 | 101 |
| | | | |
| Serum 3 | 0 | 3.5 | |
| | 4.7 | 8.2 | 99 |
| | 9.4 | 12.6 | 97 |
| | 18.8 | 22.1 | 99 |

| Sample | Spiked Concentration of IL-6 pg/mL | Concentration observed in the assay (pg/mL) | Recovery% |
|---|---|---|---|
| Serum 4 | 0 | 0.4 | |
| | 4.7 | 4.8 | 93 |
| | 9.4 | 9.2 | 94 |
| | 18.8 | 18.0 | 94 |
| | | | |
| Serum 5 | 0 | 0.3 | |
| | 4.7 | 4.6 | 91 |
| | 9.4 | 8.9 | 91 |
| | 18.8 | 17.5 | 91 |
| | | | |
| Average | | | 93 |

| Sample | Spiked Concentration of IL-6 pg/mL | Concentration observed in the assay (pg/mL) | Recovery% |
|---|---|---|---|
| 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 |
| | | | |
| Average | | | 103 |

## ASSAY CHARACTERISTICS (continued)

### E. Linearity of Dilution

10 spiked individual human serum and plasma samples were assayed for linearity studies. Sample volumes of 50 µL, 25 µL, 12.5 µL, and 6.25 µL represents dilution factors of neat, 2, 4, and 8, respectively. Required amounts of serum matrix were added to compensate for the lost volumes below 50 µL.

Dilution linearity= (observed/expected) x 100%
Observed= mean calculated dilution corrected concentration at each dilution
Expected = mean calculated concentration of the neat sample

| Sample | Sample volume (µL) | Mean (pg/mL) | Dilution Corrected (pg/mL) | Linearity% |
|---|---|---|---|---|
| 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 |
| | | | | |
| Average | | | | 110 |

| Sample | 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.

## QUALITY CONTROLS

The ranges for Quality Control 1, 2, and 3 are provided on the card insert or can be located at the MILLIPORE SIGMA website www.milliporesigma.com.

## TROUBLESHOOTING GUIDE

1. 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.
2. Throughout the assay, the operator should adhere strictly to the procedures with good laboratory practice.
3. 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.
4. Avoid cross-contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. 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.
7. Remove any air bubbles formed in the well after acidification of the substrate solution because bubbles interfere with spectrophotometric readings.
8. High signal in the background or blank wells could be due to 1.) cross well contamination by standard solution or sample or 2.) inadequate washing of wells with Wash Buffer or 3.) overexposure to light after the substrate has been added.

## ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist. Contact information for each region can be found on our website:

emdmillipore.com/contact

### Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

### Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.