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# Quantikine<sup>®</sup> HS

## HIGH SENSITIVITY

human  
IL-6

FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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## INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic cytokine that plays a central role in the network of cytokines that maintain homeostasis (1 - 3). The human IL-6 cDNA sequence predicts a protein of 212 amino acids (aa) (4 - 7). Cleavage of a signal peptide produces a mature protein of 184 aa with 4 cysteines, 2 N-linked glycosylation sites and a predicted mass of 21 kDa. While IL-6 appears to be glycosylated, glycosylation is not necessary for bioactivity (3). Mouse and rat IL-6 have about 40% sequence identity with human IL-6, but neither has N-linked glycosylation sites (8, 9). Human IL-6 is active with mouse cells, but mouse IL-6 exhibits no activity with human cells (10). Sequence and gene-structure similarities indicate that IL-6 is in a cytokine family that includes OSM, IL-11, G-CSF, LIF, CNTF, and cardiotrophin-1 (11, 12). Each of these is predicted to have a four-helix-bundle structure similar to that of growth hormone, suggesting a common ancestral gene (13 - 15).

The receptor for IL-6 (IL-6R) is a highly glycosylated, 80-kDa transmembrane protein (16). The intracellular part of the receptor has no obvious signaling activity. Rather, the receptor-IL-6 complex signals by associating with a separate transmembrane signaling protein, gp130, (17, 18) which serves a similar function for other receptors as well. Soluble forms of both IL-6R and gp130 have been found in blood (19, 20). It appears that IL-6 bound to sIL-6R is an agonist, presumably complexing with membrane-associated gp130 to deliver a signal; in contrast, sgp130 is an antagonist of IL-6 activity, competing with membrane-associated gp130 (20, 21).

IL-6 plays a key role in virtually any response to a challenge to homeostasis (1 - 3). It is a major mediator of the acute phase reaction; it stimulates differentiation and antibody secretion by B cells; it potentiates phytohemagglutinin activation of T cell production of IL-2 and IL-2R; it enhances the IL-2 and IFN- $\gamma$  induction of differentiation of cytotoxic T cells; it is a hematopoietic growth factor; it contributes to bone remodeling; and it induces neuronal cell differentiation (1 - 3, 22, 23). While IL-6 is usually considered an inflammatory cytokine, it has important anti-inflammatory functions as well (24). The concentration of IL-6 in serum is about 1 pg/mL in healthy subjects, however, it is elevated by general trauma, endotoxemia, acute or chronic inflammation, infectious or metastatic diseases, or by nearly anything that triggers a host defense response (25, 26).

Current methods for bioassay of this interleukin are based on the mitogenic effects of IL-6 on appropriate cell lines, such as B9, a mouse B cell hybridoma line, or T1165.85.2.1, a mouse plasmacytoma cell line. These bioassays are time-consuming and are not completely specific for IL-6. The Quantikine HS IL-6 Immunoassay is a solid phase ELISA that specifically measures human IL-6 in 21.5 - 27.5 hours (with an overnight incubation step). This kit is designed to measure IL-6 levels in serum, plasma, and urine. With the increased sensitivity of this kit, it is now possible to measure the levels of IL-6 present in samples from normal subjects. It contains *E. coli*-expressed recombinant human IL-6 and antibodies raised against recombinant human IL-6 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural IL-6 showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that the Quantikine HS Immunoassay kit can be used to determine relative mass values for natural IL-6. Using the Quantikine HS kit, the measurement of IL-6 is insensitive to the addition of the recombinant form of the IL-6 soluble receptor. Therefore it is probable that experimental sample measurements reflect the total amount of IL-6 present, *i.e.*, the total amount of free IL-6 plus the amount of IL-6 initially bound to soluble receptors, if any are present in the samples. A group has reported high levels of high-affinity autoantibodies to IL-6 in the serum of some normal blood donors (27, 28). Such autoantibodies would have the potential to interfere with the measurement of IL-6 by ELISAs.

## PRINCIPLE OF THE ASSAY

### DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

## ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## AMPLIFICATION SYSTEM

The Quantikine HS Immunoassay kit uses an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle leading to the formation of a colored product (29 - 31). In this amplification system, alkaline phosphatase dephosphorylates the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH (Substrate), to reduced nicotinamide adenine dinucleotide, NADH. The NADH subsequently serves as a specific cofactor that activates a redox cycle driven by the secondary enzyme system consisting of alcohol dehydrogenase and diaphorase (Amplifier). In the reaction catalyzed by diaphorase, NADH reduces a tetrazolium salt (INT-violet or iodinitrotetrazolium violet) to produce an intensely colored formazan dye and NAD<sup>+</sup>. NAD<sup>+</sup> in turn is reduced by ethanol, in an alcohol dehydrogenase-catalyzed reaction, to regenerate NADH which can then re-enter the redox cycle. The rate of reduction of the tetrazolium salt and thus the amount of colored product formed are directly proportional to the amount of IL-6 bound in the initial step.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- Although this kit has been designed to eliminate matrix problems, some samples may exist that give falsely elevated values when assayed neat. If serum, plasma or urine samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

## REAGENTS

**IL-6 Microplate** (Part 890135) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against IL-6.

**IL-6 Conjugate** (Part 890136) - 21 mL of polyclonal antibody against IL-6, conjugated to alkaline phosphatase, with preservative.

**IL-6 Standard** (Part 890137) - 50 pg of recombinant human IL-6 in a buffered protein base with preservative, lyophilized.

**Assay Diluent HD1D** (Part 895061) - 6 mL of a buffered protein base with preservative. Contains a precipitate.

**Calibrator Diluent HD5B** (Part 895065) - 21 mL of a buffered protein base with preservative.  
*For urine samples.*

**Calibrator Diluent HD6F** (Part 895069) - 21 mL of animal serum with preservative.  
*For serum/plasma samples.*

**Wash Buffer Concentrate** (Part 895073) - 100 mL of a 5-fold concentrated solution of buffered surfactant with preservative.

**Substrate** (Part 895077) - Lyophilized NADPH with stabilizers.

**Substrate Diluent** (Part 895078) - 7 mL of buffered solution with stabilizers.

**Amplifier** (Part 895075) - Lyophilized amplifier enzymes with stabilizers.

**Amplifier Diluent** (Part 895076) - 7 mL of buffered solution containing ethanol and INT-violet with stabilizers.

**Stop Solution** (Part 895074) - 6 mL of 2 N sulfuric acid.

**Plate Covers** - 8 adhesive strips.

## STORAGE

|   |  |   |
|---|--|---|
| <b>Unopened Kit</b>                           | Store at 2 - 8° C. Do not use past kit expiration date.  |   |
| <b>Opened/<br/>Reconstituted<br/>Reagents</b> | Diluted Wash Buffer  | Store for up to 1 month at 2 - 8° C.*   |
|   | Stop Solution  |   |
|   | Assay Diluent HD1D   |   |
|   | Calibrator Diluent HD5B  |   |
|   | Calibrator Diluent HD6F  |   |
|   | Conjugate  | Aliquot and store for up to 1 month at ≤ -20°C. Avoid repeated freeze-thaw cycles.* |
|   | Standard   |   |
|   | Substrate Solution   |   |
| Amplifier Solution                            | Store for up to 1 month at ≤ -20° C. Avoid repeated freeze-thaw cycles.*   |   |
| Microplate Wells                              | Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.* |   |

\*Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, with dual wavelength correction (correction wavelength set at 650 nm or 690 nm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, squirt bottle, manifold dispenser or automated microplate washer.
- Graduated cylinders: 100 mL and 500 mL for preparation of Wash Buffer.

## PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

This kit contains Thimerosal, a mercury containing compound. The total amount of mercury in this kit is 13.3 mg.

## SAMPLE COLLECTION AND STORAGE

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

*Note: The use of heparinized plasma is not recommended.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

## REAGENT PREPARATION

Bring all reagents to room temperature before use.

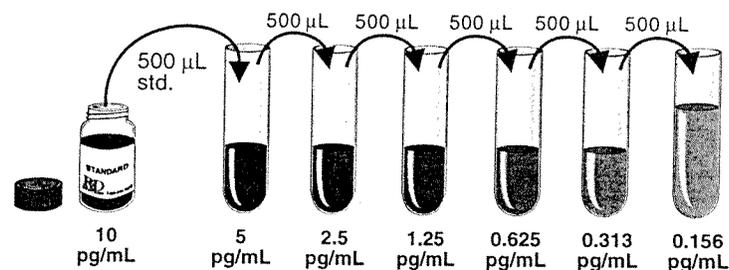
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Reconstitute the lyophilized Substrate in 6 mL of Substrate Diluent at least 10 minutes before use. Mix thoroughly. **Discard the rubber stopper after reconstitution.** Avoid contamination.

**Amplifier Solution** - Reconstitute the lyophilized Amplifier in 6 mL of Amplifier Diluent at least 10 minutes before use. Mix thoroughly. **Discard the rubber stopper after reconstitution.**

**IL-6 Standard** - Reconstitute the IL-6 Standard with 5 mL of the appropriate Calibrator Diluent (Calibrator Diluent HD6F for serum/plasma samples or Calibrator Diluent HD5B for urine samples). This reconstitution produces a stock solution of 10 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500  $\mu\text{L}$  of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (10 pg/mL). The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



\* For 8 plates 3.2 mL required for 15 fd  
pts : 2 mL diluent 4 mL 4 mL  
Save extra 10 pg/mL.

## ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 50  $\mu$ L of Assay Diluent HD1D to each well. Assay Diluent HD1D contains a precipitate. Mix well before and during its use.
4. Add 200  $\mu$ L of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 14 - 20 hours at room temperature.
5. Wash

### Notes on washing

- Excessive drying of the wells can lead to poor assay performance and imprecision. Subsequent reagents should be added immediately after washing the plate, and the wells not allowed to completely dry. Also avoid prolonged exposure of the wells to vacuum aspiration apparatus.
- After removal of the adhesive strip prior to washing, the strip can be adhered to the underside of the wells to avoid the possibility of wells becoming dislodged during the decanting and rapping operations.
- Inclusion of a 30 second soak between each addition of Wash Buffer and decanting of the plate contents will improve the precision of the assay.

### Wash Procedure

- a. Remove liquid from the wells by inverting the plate and decanting the contents.
  - b. Remove excess liquid by grasping the plate firmly and smartly rapping the plate inverted on a clean paper towel at least 5 times.
  - c. Fill each well with 400  $\mu$ L of Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher.
  - d. Remove liquid from the wells by inverting the plate and decanting the contents.
  - e. Repeat steps b - d three times for a total of 4 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200  $\mu$ L of IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 6 hours at room temperature.
  7. Repeat the wash as in step 5.
  8. Add 50  $\mu$ L of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 60 minutes at room temperature. **Do not wash the plate.**
  9. Add 50  $\mu$ L of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.  
Note: Addition of Amplifier Solution initiates color development.
  10. Add 50  $\mu$ L of Stop Solution to each well. Addition of Stop Solution does not affect color in the wells.
  11. Determine the optical density of each well within 30 minutes using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

## ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as instructed.



2. Add 50  $\mu$ L Assay Diluent HD1D to each well.



3. Add 200  $\mu$ L Standard or sample to each well. Incubate 14-20 hrs. RT *overnight*



4. Wash 4 times.



5. Add 200  $\mu$ L Conjugate to each well. Incubate 6 hrs. RT *8 AM  $\rightarrow$  2 PM*



6. Wash 4 times.



7. Add 50  $\mu$ L Substrate Solution to each well. Incubate 60 min. RT



8. Add 50  $\mu$ L Amplifier Solution to each well. Incubate 30 min. RT



9. Add 50  $\mu$ L Stop Solution to each well. Read at 490 nm within 30 min.  $\lambda$  correction 650 or 690 nm

## CALCULATION OF RESULTS

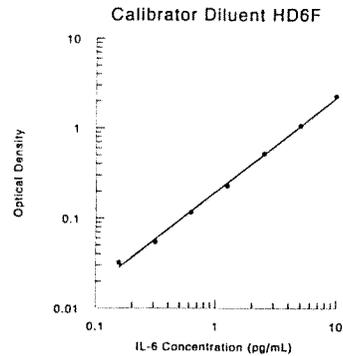
Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Plot the optical density of the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

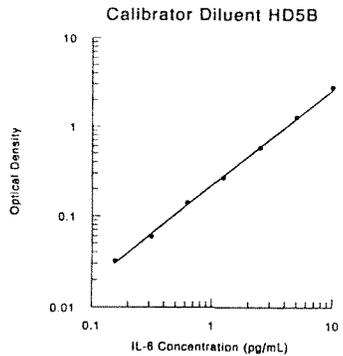
To determine the IL-6 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding IL-6 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL STANDARD CURVES

The following standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



| (pg/mL) | O.D.                    | Average | Corrected |
|---------|-------------------------|---------|-----------|
| 0       | 0.047<br>0.049<br>0.084 | 0.048   |           |
| 0.156   | 0.077<br>0.105          | 0.080   | 0.032     |
| 0.313   | 0.100<br>0.181          | 0.102   | 0.054     |
| 0.625   | 0.148<br>0.289          | 0.164   | 0.116     |
| 1.25    | 0.265<br>0.581          | 0.277   | 0.229     |
| 2.5     | 0.557<br>1.147          | 0.569   | 0.521     |
| 5       | 1.059<br>2.305          | 1.103   | 1.055     |
| 10      | 2.312                   | 2.308   | 2.260     |



| (pg/mL) | O.D.                    | Average | Corrected |
|---------|-------------------------|---------|-----------|
| 0       | 0.042<br>0.044<br>0.075 | 0.043   |           |
| 0.156   | 0.076<br>0.102          | 0.076   | 0.032     |
| 0.313   | 0.102<br>0.180          | 0.102   | 0.059     |
| 0.625   | 0.186<br>0.317          | 0.183   | 0.140     |
| 1.25    | 0.306<br>0.613          | 0.312   | 0.268     |
| 2.5     | 0.629<br>1.437          | 0.621   | 0.578     |
| 5       | 1.190<br>3.024          | 1.314   | 1.271     |
| 10      | 2.596                   | 2.810   | 2.767     |

## TECHNICAL HINTS

- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as the Substrate Solution.
- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

## PRECISION

### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were assayed in replicates of twenty to assess intra-assay precision.

### Inter-assay Precision (Precision between assays)

Three samples of known concentration were evaluated in twenty separate assays to assess inter-assay precision.

### Serum Assay

| Sample             | Intra-assay Precision |      |      | Inter-assay Precision |       |      |
|--------------------|-----------------------|------|------|-----------------------|-------|------|
|                    | 1                     | 2    | 3    | 1                     | 2     | 3    |
| n                  | 20                    | 20   | 20   | 20                    | 20    | 20   |
| Mean (pg/mL)       | 0.36                  | 2.73 | 7.94 | 0.561                 | 3.575 | 8.16 |
| Standard Deviation | 0.04                  | 0.16 | 0.30 | 0.09                  | 0.59  | 0.81 |
| CV (%)             | 11.1                  | 5.9  | 3.8  | 16.0                  | 16.5  | 9.9  |

### Urine Assay

| Sample             | Intra-assay Precision |      |      | Inter-assay Precision |      |      |
|--------------------|-----------------------|------|------|-----------------------|------|------|
|                    | 1                     | 2    | 3    | 1                     | 2    | 3    |
| n                  | 20                    | 20   | 20   | 20                    | 20   | 20   |
| Mean (pg/mL)       | 0.49                  | 3.03 | 8.49 | 0.64                  | 3.44 | 7.42 |
| Standard Deviation | 0.03                  | 0.17 | 0.34 | 0.09                  | 0.35 | 0.69 |
| CV (%)             | 6.1                   | 5.6  | 4.0  | 14.1                  | 10.2 | 9.3  |

## RECOVERY

The recovery of IL-6 was determined in various matrices by mixing high dose samples with low dose samples in ratios of 1:2, 1:1, and 2:1.

| Sample Type    | Average % Recovery | Range      |
|----------------|--------------------|------------|
| Serum          | 110                | 100 - 123% |
| EDTA plasma    | 103                | 90 - 112%  |
| Citrate plasma | 109                | 98 - 132%  |
| Urine          | 86                 | 70 - 100%  |

## LINEARITY

To assess linearity of the assay, the following biological samples, containing or spiked with high concentrations of IL-6, were diluted with the appropriate Calibrator Diluent and then assayed.

|     |                    | Serum   | EDTA plasma | Citrate plasma | Urine   |
|-----|--------------------|---------|-------------|----------------|---------|
| 1:2 | Average % Recovery | 102     | 104         | 97             | 95      |
|     | Range (%)          | 93-104  | 97-113      | 80-108         | 91-102  |
| 1:4 | Average % Recovery | 104     | 96          | 110            | 108     |
|     | Range (%)          | 98-108  | 85-110      | 96-129         | 106-111 |
| 1:8 | Average % Recovery | 111     | 104         | 137            | 112     |
|     | Range (%)          | 103-127 | 99-109      | 125-149        | 111-113 |

## SENSITIVITY

The minimum detectable dose of IL-6 is typically less than 0.094 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-6 produced at R&D Systems.

The NIBSC/WHO IL-6 International Reference Standard 89/548, which was intended as a potency standard, was evaluated in this kit. This standard is a CHO-derived recombinant human IL-6. Each ampule contains a nominal 1 µg of glycosylated recombinant human IL-6 and was assigned a unitage of 100,000 International Units/ampule.

The dose response curve of this International Reference Standard parallels the Quantikine HS standard curve. To convert sample values obtained with the Quantikine HS IL-6 kit to equivalent NIBSC 89/548 mass values, use the equation below.

NIBSC (89/548) equivalent value (IU/mL) = 0.131 x Quantikine HS IL-6 value (pg/mL)

## SAMPLE VALUES

Serum, plasma and urine samples were evaluated in this assay.

| Sample Type           | Range (pg/mL) | % Detectable | Mean of Detectable (pg/mL) |
|-----------------------|---------------|--------------|----------------------------|
| Serum (n=72)          | 0.378 - 10.1  | 100          | 1.62                       |
| EDTA plasma (n=76)    | ND - 11.5     | 97           | 1.34                       |
| Citrate plasma (n=76) | 0.204 - 7.84  | 100          | 1.27                       |
| Urine (n=60)          | ND - 10.0     | 78           | 1.14                       |

ND = non-detectable

## SPECIFICITY

This assay recognizes both natural and recombinant human IL-6. Preparations of the following factors at 10 ng/mL in a mid-range rhIL-6 control prepared in Calibrator Diluents HD6F and HD5B were assayed. No significant cross-reactivity or interference was observed.

### Factors related to or associated with IL-6:

rhIL-6 sR                      rmlL-6

#### Other factors:

| Recombinant human: |               |                |                           |                   |
|--------------------|---------------|----------------|---------------------------|-------------------|
| IL-1 $\alpha$      | CNTF          | MCP-1          | TNF- $\beta$              | MIP-1 $\beta$     |
| IL-1 $\beta$       | $\beta$ -ECGF | MIP-1 $\alpha$ | sTNF RI                   | SCF               |
| IL-1ra             | EGF           | MIP-1 $\beta$  | sTNF RII                  | TNF- $\alpha$     |
| IL-2               | Epo           | OSM            | <b>Recombinant mouse:</b> | mEGF              |
| IL-3               | FGF acidic    | PDGF-AA        | IL-1 $\alpha$             | bFGF acidic       |
| IL-4               | FGF basic     | PDGF-AB        | IL-1 $\beta$              | bFGF basic        |
| IL-5               | FGF-4         | PDGF-BB        | IL-3                      | hPDGF             |
| IL-7               | G-CSF         | RANTES         | IL-4                      | pPDGF             |
| IL-8               | GM-CSF        | SCF            | IL-5                      | hTGF- $\beta$ 1   |
| IL-9               | GRO $\alpha$  | SLPI           | IL-7                      | pTGF- $\beta$ 1   |
| IL-10              | IFN- $\gamma$ | TGF- $\beta$ 1 | IL-9                      | pTGF- $\beta$ 1.2 |
| IL-11              | IGF-I         | TGF- $\beta$ 2 | IL-12                     | pTGF- $\beta$ 2   |
| ANG                | IGF-II        | TGF- $\beta$ 3 | GM-CSF                    | rcTGF- $\beta$ 3  |
|                    | LIF           | TGF- $\alpha$  | MIP-1 $\alpha$            | raTGF- $\beta$ 5  |
|                    | M-CSF         | TNF- $\alpha$  |                           |                   |

## APPLICABLE PATENTS

These products are covered by the following patents:

**Substrate** (Part 895077) - Lyophilized NADPH with stabilizers.

**Substrate Diluent** (Part 895078) - 7 mL of buffered solution with stabilizers.

**Amplifier** (Part 895075) - Lyophilized amplifier enzymes with stabilizers.

**Amplifier Diluent** (Part 895076) - 7 mL of buffered solution containing ethanol and INT-violet with stabilizers.

|                   |           |
|-------------------|-----------|
| <b>US:</b>        | 4,446,231 |
|                   | 4,595,655 |
|                   | 4,598,042 |
| <b>EUROPE:</b>    | 60,123    |
|                   | 27,036    |
| <b>CANADA:</b>    | 1,170,179 |
| <b>AUSTRALIA:</b> | 544,496   |

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