PRODUCT INFORMATION & MANUAL

Human sIL-6 Instant ELISA

BMS213INST

Enzyme-linked immunosorbent assay for quantitative detection of human sIL-6.

For research use only.
Not for diagnostic or therapeutic procedures.
128 Tests

eBioscience®
An Affymetrix Company

Human sIL-6 Instant ELISA

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
europe@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

TABLE OF CONTENTS

1 Intended Use

The human IL-6 Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-6. **The human IL-6 Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms. The gene for human IL-6 has been localized to chromosome 7p21. The genomic sequence has been determined. IL-6 is usually not produced constitutively by normal cells, but its expression is readily induced by a variety of cytokines, lipopolysaccharide or viral infections. The IL-6 gene product is a single chain protein with a molecular mass ranging from 21 to 28 kDa, depending on the cellular source. Extensive posttranslational modifications like N- and O-linked glycosylation as well as phosphorylation seem to account for this heterogeneity. The cDNA for IL-6 predicts a precursor protein of 212 amino acids.
IL-6 is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation respectively, depending on the nature of the target cells.

IL-6 is involved in
- the induction of B-cell differentiation,
- the induction of acute phase proteins in liver cells,
- growth promotion of myeloma/plasmacytoma/hybridoma cells,
- induction of IL-2 and IL-2 receptor expression,
- proliferation and differentiation of T cells,
- inhibition of cell growth of certain myeloid leukemic cell lines and induction of their differentiation to macrophages,
- enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of maturation of megakaryocytes as a thrombopoietic factor,
- induction of mesangial cell growth,
- induction of neural differentiation of PC 12 cells and
- induction of keratinocyte growth.

The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with autoantibody production in patients with cardiac myxoma. Since then, IL-6 has been suggested to be involved in

the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations.

Infections:
Body fluids of patients with acute local bacterial or viral infections and serum of patients with gram-negative or positive bacteremia contain elevated levels of biologically active IL-6.

Obstetric Infections:
IL-6 has emerged as a reporter cytokine for intraamniotic infection.

Diseases associated with an altered immune system (polyclonal B-cell abnormalities or autoimmune diseases):
Elevated levels of circulating IL-6 have been detected in patients with cardiac myxoma, Castleman's disease, rheumatoid arthritis, IgM gammopathy and in those with acquired immunodeficiency syndrome as well as alcoholic liver cirrhosis.

Proliferative diseases:
Elevated plasma levels of IL-6 are observed in patients with psoriasis and mesangial proliferative glomerulonephritis.

Neoplastic Diseases:
Increased systemic levels of IL-6 have been detected in patients with multiple myeloma, other B-cell dyscrasias, Lennert's T lymphoma, Castleman's disease, renal cell carcinoma and various other solid tumors.

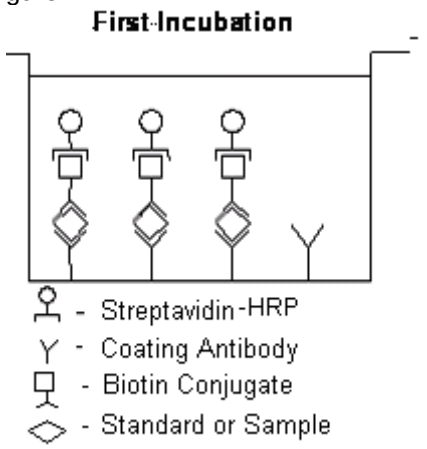
Inflammatory responses:
IL-6 is involved in the induction of acute phase proteins and induction of fever. Elevated serum levels of IL-6 are also found in patients with severe burns, in serum and plasma as a marker for predicting postoperative complications, in serum and urine of recipients of kidney transplants before rejection, in the serum of septic shock patients and in patients with inflammatory arthritis and traumatic arthritis.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

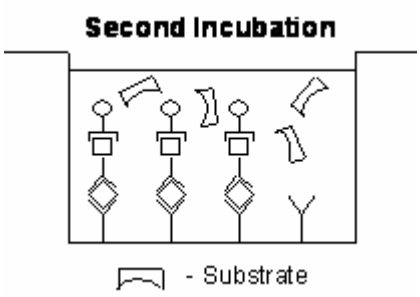
An anti-human IL-6 coating antibody is adsorbed onto microwells. Human IL-6 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human IL-6 antibody binds to human IL-6 captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human IL-6.

Figure 1



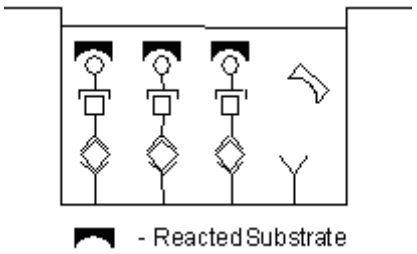
Following incubation unbound biotin conjugated antihuman IL-6 and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human IL-6 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-6 standard dilutions and human IL-6 sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human IL-6, **Biotin-Conjugate** (anti-human IL-6 monoclonal antibody), **Streptavidin-HRP** and Sample Diluent, lyophilized

2 aluminium pouches with a human IL-6 **Standard curve** (coloured)

1 bottle (25 ml) **Wash Buffer Concentrate** 20x (phosphate-buffered saline with 1% Tween 20)

1 vial (12 ml) **Sample Diluent**
(Use when an external predilution of the samples is needed)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

1 vial **Control high,** lyophilized

1 vial **Control low,** lyophilized

2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate, standard curves and controls or whole kit at -20°C. The plate, the standard curves and the controls can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant and serum were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human IL-6. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 µl and 500 µl) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

Buffer concentrate should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in the **buffer concentrate**, warm it gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer is stable for 30 days.

9.2 Controls

Solubilize by adding 300 µl distilled water to lyophilized **controls.** Swirl or mix gently to ensure complete and homogeneous solubilization. Allow the controls to reconstitute for 10-30 minutes. Mix well prior to further use.
Treat the controls like your samples in the assay. For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
- **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
- **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
- **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
- **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
- **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**

a. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (coloured). Each sample, standard, blank and control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

c. Add 100 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (200.0 pg/ml)	Standard 1 (200.0 pg/ml)	Sample 1	Sample 1
B	Standard 2 (100.0 pg/ml)	Standard 2 (100.0 pg/ml)	Sample 2	Sample 2
C	Standard 3 (50.0 pg/ml)	Standard 3 (50.0 pg/ml)	Sample 3	Sample 3
D	Standard 4 (25.0 pg/ml)	Standard 4 (25.0 pg/ml)	Sample 4	Sample 4
E	Standard 5 (12.5 pg/ml)	Standard 5 (12.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (6.3 pg/ml)	Standard 6 (6.3 pg/ml)	Sample 6	Sample 6
G	Standard 7 (3.1 pg/ml)	Standard 7 (3.1 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 50 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.

e. Cover with an adhesive film and incubate at room temperature (18ºC to 25ºC) for 3 hours, if available on a microplate shaker at 400 rpm.

f. Remove adhesive film and empty wells. **Wash** the microwell strips 6 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

   It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

j. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human IL-6 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-6 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human IL-6 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-6 concentration.

- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human IL-6 levels. Such samples require further external predilution according to expected human IL-6 values with Sample Diluent in order to precisely quantitate the actual human IL-6 level.**

- It is suggested that each testing facility establishes a control sample of known human IL-6 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 4

Representative standard curve for human IL-6 Instant ELISA. Human IL-6 was diluted in serial 2-fold steps in Sample Diluent. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

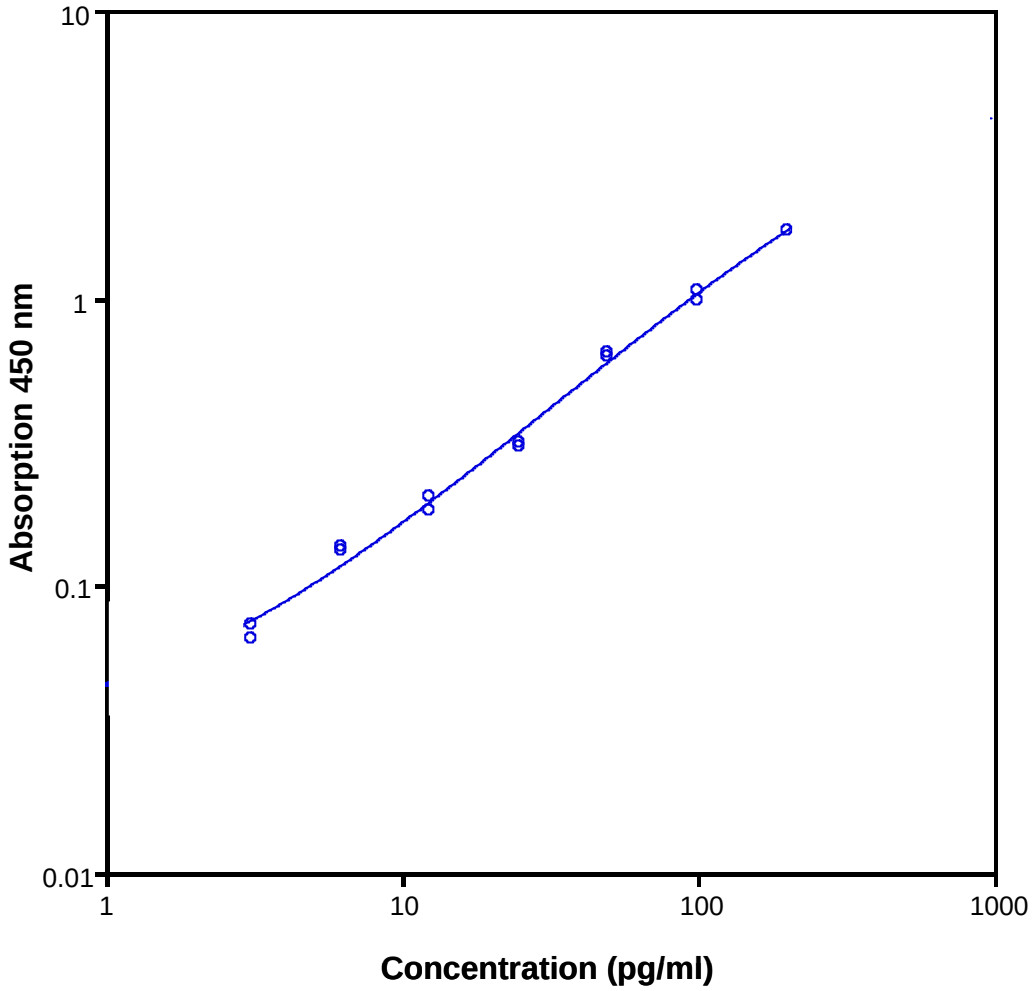


Table 2
Typical data using the human IL-6 INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human IL-6 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	200.0	1.708	1.715	5.2
		1.721		
2	100.0	1.058	1.019	3.6
		0.981		
3	50.0	0.625	0.635	2.2
		0.645		
4	25.0	0.313	0.309	5.1
		0.304		
5	12.5	0.205	0.194	8.2
		0.182		
6	6.3	0.136	0.134	6.4
		0.132		
7	3.1	0.073	0.069	6.8
		0.065		
Blank	0.0	0.041	0.037	10.8
		0.033		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human IL-6 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.92 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-6. 2 standard curves were run on each plate. Data below show the mean human IL-6 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.2%.

Table 3

The mean human IL-6 concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human IL-6 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	100.5	4
	2	111.4	8
	3	110.2	5
2	1	59.2	9
	2	53.6	7
	3	56.1	6
3	1	30.1	5
	2	28.3	5
	3	32.2	3
4	1	168.9	8
	2	166.6	6
	3	148.0	4
5	1	59.0	5
	2	52.5	8
	3	56.5	7
6	1	27.1	8
	2	23.1	7
	3	29.6	5

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-6. 2 standard curves were run on each plate. Data below show the mean human IL-6 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 7.0%.

Table 4
The mean human IL-6 concentration and the coefficient of variation of each sample

Sample	Mean Human IL-6 Concentration (pg/ml)	Coefficient of Variation (%)
1	107.4	5.6
2	56.3	5.0
3	30.2	6.4
4	161.2	7.1
5	56.0	5.8
6	26.6	12.3

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IL-6 into normal human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments.
Average recovery ranged from 77% to 110% with an overall mean recovery of 90%.

13.4 Dilution Parallelism

Serum samples with different levels of human IL-6 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 100% and 118% with an overall recovery of 108% (see Table 5).

Table 5

Sample	Dilution	Mean Human IL-6 Concentration (pg/ml)		% Recovery of Exp. Val.
		Expected Value	Observed Value	
1	1:2	--	665	--
	1:4	333	379	114
	1:8	166	179	108
2	1:2	--	343	--
	1:4	172	203	118
	1:8	86	86	100
3	1:2	--	657	--
	1:4	329	354	108
	1:8	164	175	106
4	1:2	--	339	--
	1:4	169	176	104
	1:8	85	92	109

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human IL-6 levels determined.
There was no significant loss of human IL-6 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-6 level determined after 24 h.
There was no significant loss of human IL-6 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The interference of circulating factors of the immune syteme was evaluated by spiking these proteins at physiologically relevant concentrations into a human IL-6 positive serum.
There was no crossreactivity detected.

13.7 Expected Values

A panel of 40 serum samples from randomly selected apparently healthy donors was tested for human IL-6.
For detected human IL-6 levels see Table 6:

Table 6

Sample Matrix	Number of Samples Evaluated	Range (pg/ml)	% Detectable	Mean of Detectable (pg/ml)
Serum	40	n.d. – 12.7	47.5	5.8

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

13.8 Calibration

The immunoassay is calibrated with highly purified recombinant human IL-6 which has been evaluated against the international Reference Standard NIBSC 89/548 and has been shown to be equivalent. NIBSC 89/548 is quantitated in International Units (IU), 1IU corresponding to 10 pg human IL-6.

14 Ordering Information

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:

877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:

888.999.1371
858.642.2058
info@eBioscience.com

Fax:

858.642.2046

Europe/International*

Technical Support:

+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:

+43 1 796 40 40-304
europe@eBioscience.com

Fax:

+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

15.2 Controls

Solubilize by adding 300 µl distilled water to lyophilized **controls**.

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 µl distilled water to sample wells.
- Add 50 µl sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) if available on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 6 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.