

# *PRODUCT INFORMATION AND MANUAL*

## ***human sIL-6R Instant ELISA***

### ***BMS214INST***

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

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests

**human sIL-6R  
BMS214INST**



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## 1 Intended Use

The human sIL-6R Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of soluble human interleukin-6 receptor levels in cell culture supernatants, human serum, plasma, urine, amniotic fluid or other body fluids. The human sIL-6R Instant ELISA is **for research use only. Not for diagnostic or therapeutic procedures.**

## 2 Summary

Interleukin-6 (IL-6) is a multifunctional cytokine involved in the regulation of the immune response, hematopoiesis and acute phase response (4). It has been recognized to be a member of the  $\alpha$ -helical cytokine family (14).

IL-6 exerts its action via a cell surface receptor which consists of two subunits, an 80 kDa ligand binding subunit (gp80) of 468 amino acids and a 130 kDa signal transducing protein (gp130) of 896 amino acid residues (9, 13, 15). The cDNAs of both proteins have been cloned (5, 15). Both subunits belong to the recently recognized hematopoietic receptor superfamily which includes many cytokine receptors.

Characterisation of the extracellular portion of the 80 kDa IL-6 receptor revealed the existence of a single immunoglobulin-like domain in the NH<sub>2</sub>-terminal of the extracellular region, which does not contribute to ligand binding. The remainder of the extracellular domain however is essential for low affinity ligand binding, which consecutively triggers the association of the receptor and gp130 thus forming a high affinity binding site for IL-6 (8).

For many cytokine receptors soluble forms have been demonstrated (2). These soluble molecules have been observed to retain ligand binding capacity and therefore compete with the membrane receptors, thus acting as antagonists (2). A soluble form of the human gp80 protein has been detected in serum and urine samples (6, 12). This 55 kDa protein representing the extracellular portion of gp80 is generated by shedding, a process that seems to be controlled by protein kinase C (10). It is still functional, indicating that soluble gp80 plays a biological role in promoting IL-6 activity (5). So far, the soluble IL-6 receptor is unique in acting as an agonist together with its ligand (11).

The role of soluble IL-6R as a marker for a number of pathological situations has been demonstrated.

- HIV: sIL-6R levels are increased in patients with human immunodeficiency virus infection (6).
- Multiple myeloma (MM), monoclonal gammopathy of undertermined significance (MGUS): Serum soluble IL-6R levels are significantly increased in individuals with MGUS and in patients with MM as compared to age-related healthy individuals. These levels are independent of previously recognized prognostic factors in MM, especially serum IL-6 levels and myeloma cell mass (1, 3).
- Hodgkin's Disease (HD): Elevated levels of IL-6 receptor expression in the mixed cellularity subtype of HD have been demonstrated (7).

### 3 Principles of the Test

An anti-human sIL-6R monoclonal coating antibody is adsorbed onto microwells. Human sIL-6R present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-human sIL-6R antibody binds to human sIL-6R captured by the first antibody.

Following incubation unbound enzyme conjugated anti-human sIL-6R is removed during a wash step and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of human sIL-6R 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 seven human sIL-6R standard dilutions and human sIL-6R sample concentration determined.

Figure 1

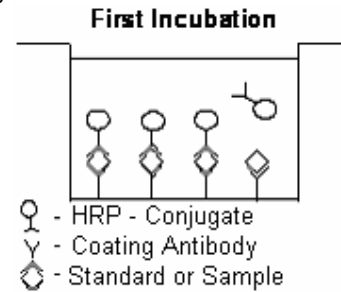


Figure 2

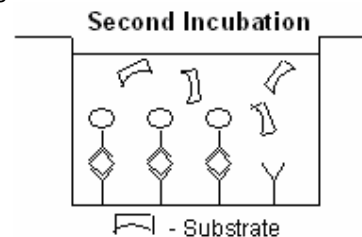
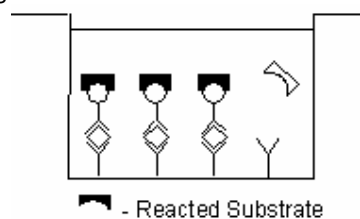


Figure 3



#### 4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated with Monoclonal Antibody** (murine) to human sIL-6R and **HRP-Conjugate** (anti-sIL-6R monoclonal (murine) antibody), lyophilized
- 2 aluminium pouches with a **human sIL-6R Standard curve (coloured)**
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (5 ml) **Assay Buffer Concentrate 20x** (PBS with 1% Tween 20 and 10% BSA). (Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 adhesive **Plate Covers**

#### 5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves 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 supernatants, human serum, plasma, urine, amniotic fluid or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells as soon as possible after clotting and separation.

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  $-20^{\circ}\text{C}$  to avoid loss of bioactive human sIL-6R. If samples are to be run within 24 hours, they may be stored at  $2^{\circ}$  to  $8^{\circ}\text{C}$  (for sample stability refer to 13).

Avoid repeated freeze-thaw cycles. Prior to assay, frozen serum or plasma should be brought to room temperature slowly and mixed gently.

## 7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5  $\mu$ l to 1000  $\mu$ l adjustable single channel micropipettes with disposable tips
- 50  $\mu$ l to 300  $\mu$ l adjustable multichannel micropipette 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 chemicals 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

### 9.1 Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (25 ml) of the Wash Buffer Concentrate 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. The pH of the final solution should adjust to 7.4.

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 Assay Buffer

Pour the entire contents (5ml) of the **Assay Buffer Concentrate** into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently.

## 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 in order that all the lyophilised pellets remain in the wells**
- a. Predilute serum or plasma samples 1:50 with Assay Buffer according to the following dilution scheme:
- 10 µl Sample + 490 µl Assay Buffer
- b. 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 optional 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).
- c. Add 130 µl of distilled water to the sample wells.
- d. Add distilled water to all standard and blank wells as indicated on the label of the standard strips (A1, A2 to H1, H2).

Table 1

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

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>A</b>	Standard 1 (5.000 ng/ml)	Standard 1 (5.000 ng/ml)	Sample 1	Sample 1
<b>B</b>	Standard 2 (2.500 ng/ml)	Standard 2 (2.500 ng/ml)	Sample 2	Sample 2
<b>C</b>	Standard 3 (1.250 ng/ml)	Standard 3 (1.250 ng/ml)	Sample 3	Sample 3
<b>D</b>	Standard 4 (0.625 ng/ml)	Standard 4 (0.625 ng/ml)	Sample 4	Sample 4
<b>E</b>	Standard 5 (0.313 ng/ml)	Standard 5 (0.313 ng/ml)	Sample 5	Sample 5
<b>F</b>	Standard 6 (0.156 ng/ml)	Standard 6 (0.156 ng/ml)	Sample 6	Sample 6
<b>G</b>	Standard 7 (0.078 ng/ml)	Standard 7 (0.078 ng/ml)	Sample 7	Sample 7
<b>H</b>	Blank	Blank	Sample 8	Sample 8

- e. Add 20 µl of each 1:50 prediluted **Sample**, in duplicate, to the designated wells and mix the contents.
- f. Cover with a **Plate Cover** and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker at 100 rpm.
- g. Remove **Plate Cover** and empty wells. Wash the microwell strips 3 times with approximately 400 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. 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.

- h. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- i. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

**The colour development on the plate should be monitored and the substrate reaction stopped (see point j. 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 0.6 – 0.65.

- j. 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.
- k. 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 sIL-6R 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 sIL-6R concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating human sIL-6R 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 sIL-6R concentration.
- **\*Samples have been diluted 1:250, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 250).**
- It is suggested that each testing facility establishes a control sample of known human sIL-6R 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.

\* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. These 100 µl are composed of 80 µl of sample diluent plus 20 µl of the 1:50 prediluted sample. This is a 1:250 dilution.

The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.

80 µl sample diluent and 50 µl conjugate results in 130 µl reconstitution volume, addition of 20 µl 1:50 prediluted sample (80 µl + 20 µl 1:50 prediluted sample = 1:250 dilution)

Figure 4

Representative standard curve for human sIL-6R Instant ELISA. Human sIL-6R was diluted in serial 2-fold steps in Assay Buffer, 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.

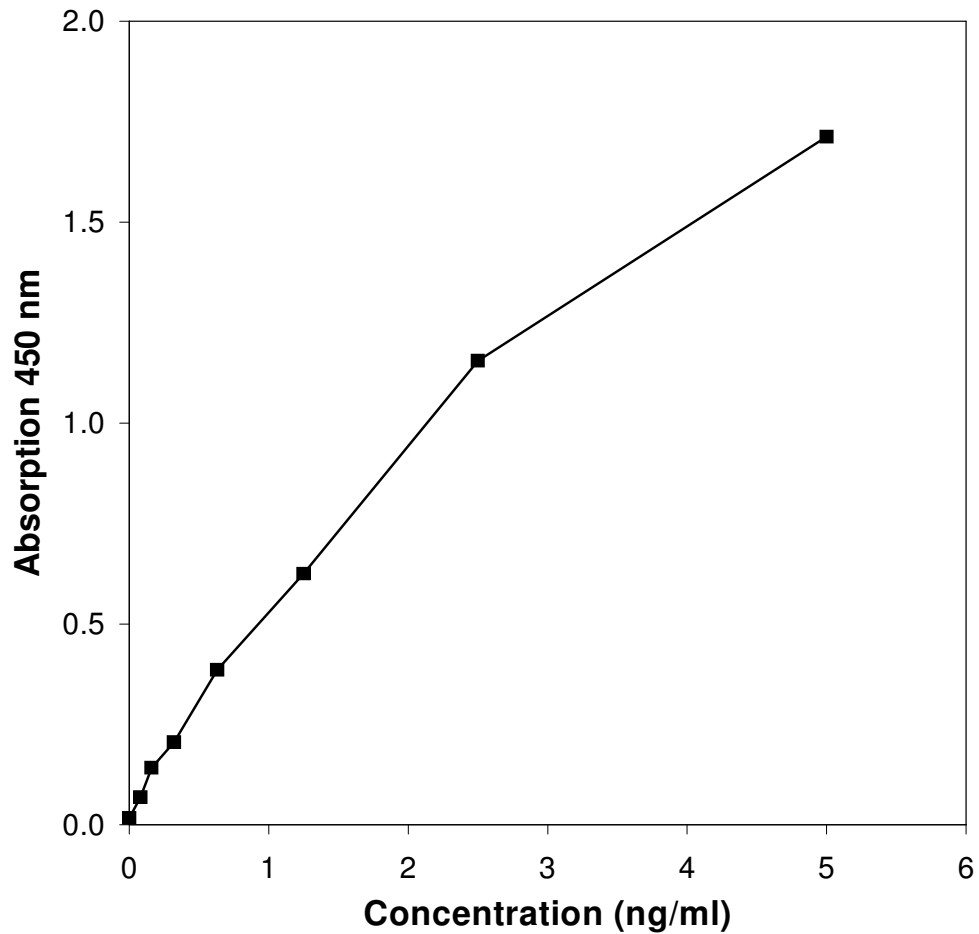


Table 2

Typical data using the human sIL-6R INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human sIL-6R Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	5.000	1.803	1.713	7.4
	5.000	1.623		
2	2.500	1.135	1.155	2.4
	2.500	1.175		
3	1.250	0.665	0.652	2.8
	1.250	0.639		
4	0.625	0.386	0.386	0.0
	0.625	0.386		
5	0.313	0.215	0.206	5.8
	0.313	0.198		
6	0.156	0.134	0.142	7.0
	0.156	0.148		
7	0.078	0.069	0.069	0.0
	0.078	0.069		
Blank	0	0.016	0.017	
	0	0.018		

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 sIL-6R 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 0.010 ng/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 8 serum samples containing different concentrations of human sIL-6R. 2 standard curves were run on each plate. Data below show the mean human sIL-6R concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.8%.

Table 3

The Mean human sIL-6R concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	human sIL-6R Concentration (ng/ml)	Coefficient of Variation (%)
1	1	74.3	6
	2	84.9	8
	3	83.2	8
2	1	109.7	9
	2	111.7	6
	3	104.4	8
3	1	105.8	4
	2	112.0	5
	3	114.3	4
4	1	96.7	9
	2	104.0	8
	3	83.0	2
5	1	101.9	8
	2	93.8	1
	3	85.1	5
6	1	105.2	8
	2	111.1	9
	3	88.7	10
7	1	104.7	7
	2	101.1	1
	3	85.6	10
8	1	97.5	10
	2	108.2	9
	3	87.8	9

### 13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human sIL-6R. 2 standard curves were run on each plate. Data below (see Table 4) show the mean human sIL-6R concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 8.4%.

Table 4

The mean human sIL-6R concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	human sIL-6R Concentration (ng/ml)	Coefficient of Variation (%)
1	80.8	7.1
2	108.6	3.5
3	110.7	4.0
4	94.5	11.3
5	93.6	9.0
6	101.7	11.4
7	97.1	10.4
8	97.8	10.5

### 13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sIL-6R into human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. The overall mean recovery has been determined to be 93%.

### 13.4 Dilution Parallelism

4 serum samples with different levels of human sIL-6R were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 90% to 116% with an overall mean recovery of 102% (see Table 5).

Table 5

Sample	Human sIL-6R Concentration (ng/ml)		% Recovery of Exp. Val.	
	Dilution	Expected Value		Observed Value
1	1:250	-	306	-
	1:500	153	176	115
	1:1000	76	86	113
	1:2000	38	36	95
2	1:250	-	361	-
	1:500	181	195	108
	1:1000	90	81	90
	1:2000	45	40	89
3	1:250	-	337	-
	1:500	169	195	115
	1:1000	84	98	116
	1:2000	42	43	101
4	1:250	-	389	-
	1:500	195	210	108
	1:1000	97	80	83
	1:2000	49	45	93

### 13.5 Sample Stability

#### 13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20 °C and thawed 5 times, and the human sIL-6R levels determined. There was no significant loss of human sIL-6R immunoreactivity between 0 and 5 freeze-thaw cycles.

#### 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 sIL-6R level determined after 24 h. There was no significant loss of human sIL-6R immunoreactivity during storage under above conditions.

### **13.6 Comparison of Serum and Plasma**

From 22 individuals, serum as well as EDTA, citrate, and heparin plasma obtained at the same time point were evaluated. All these blood preparations are suitable for human sIL-6R determinations. It is nevertheless highly recommended to assure the uniformity of blood preparations.

### **13.7 Specificity**

To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed. Notably there was no influence on the human sIL-6R levels measured by the addition of human IL-6.

### **13.8 Expected Values**

A panel of 22 randomly selected sera from apparently healthy blood donors (male and female) was tested for human sIL-6R. The detected human sIL-6R levels ranged between 65.9 and 202.7 ng/ml with a mean level of 105.2 ng/ml and a standard deviation of 29.7 ng/ml.

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For literature update refer to [www.bendermedsystems.com/44.html](http://www.bendermedsystems.com/44.html)

## 15 Ordering Information

For orders please contact:

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**Cat.No. BMS214INST human sIL-6R INSTANT ELISA**

## **16 Reagent Preparation Summary**

### **16.1 Wash Buffer**

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

### **16.2 Assay Buffer**

Add **Assay Buffer Concentrate** 20 x (5 ml) to 95 ml distilled water

## 17 Test Protocol Summary

- Predilute serum or plasma samples with Assay Buffer 1:50
- Place standard strips in position A1/A2 to H1/H2.
- Add 130  $\mu\text{l}$  **distilled water** to sample wells.
- Add **distilled water**, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 20  $\mu\text{l}$  1:50 prediluted **Sample** to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on a microplate shaker at 100 rpm.
- Empty and wash microwell strips 3 times with 400  $\mu\text{l}$  **Wash Buffer**.
- Add 100  $\mu\text{l}$  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  $\mu\text{l}$  **Stop Solution** to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

**Note: Samples have been diluted 1:250, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 250).**