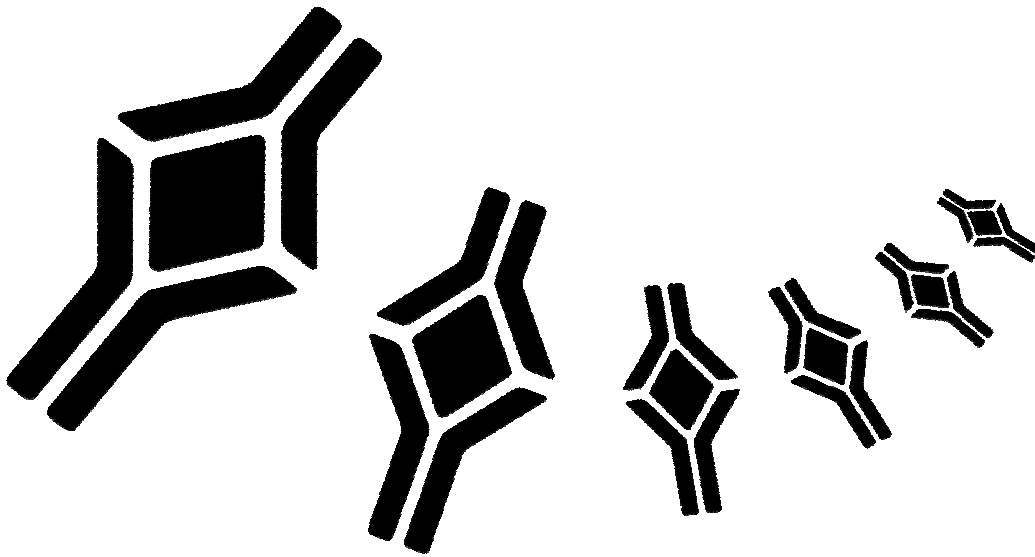


BioVendor

Research
and Diagnostic Products



HUMAN sINTERLEUKIN-6R ELISA

Product Data Sheet

Cat. No.: RBMS214R

For Research Use Only

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BioVendor – Laboratorní medicína, a.s.**

»» Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The sIL-6R ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble human interleukin-6 receptor levels in cell culture supernatants, human serum, plasma, urine, amniotic fluid or other body fluids. **The sIL-6R ELISA is for research use only. Not for use in 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 α -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 NH₂-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).

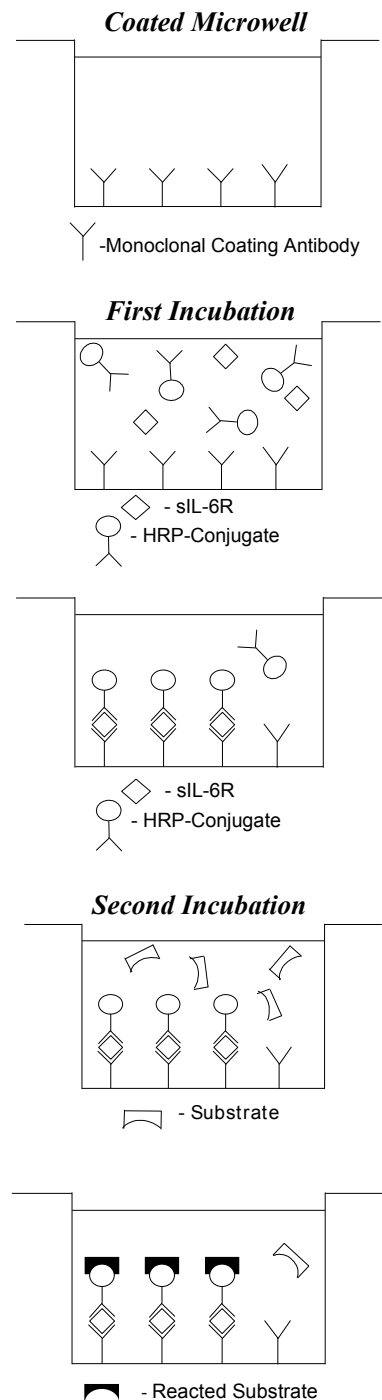
3. PRINCIPLES OF THE TEST

An anti-sIL-6R monoclonal coating antibody is adsorbed onto microwells.

sIL-6R present in the sample or standard binds to antibodies adsorbed to the microwells; a HRP-conjugated monoclonal anti-sIL-6R antibody is added and binds to sIL-6R captured by the first antibody.

Following incubation unbound enzyme conjugated anti-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 soluble IL-6R present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sIL-6R standard dilutions and sIL-6R sample concentration determined.



4. REAGENTS PROVIDED

- 1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (murine) to human sIL-6R.
- 2 vials (5 μ l) **HRP-Conjugate** anti-sIL-6R monoclonal (murine) antibody.
- 2 vials (5 ng) **sIL-6R Standard**, lyophilized.
- 1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20).
- 1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA).
- 1 vial (15ml) **Substrate Solution**
- 1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)
- 1 vial (0.4 ml each) **Blue-Dye** and **Green Dye**
- 2 adhesive **Plate Covers**

Reagent Labels

5. STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 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, EDTA, or heparinized plasma, amniotic fluid, urine or other body fluids are suitable for use in the assay. Remove serum or plasma from the clot or red cells, respectively, 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.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sIL-6R. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly and mixed gently and properly prediluted with Assay Buffer (**1:50** see 10.b.)

For stability and suitability of samples refer to 13. F and G.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1,000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ 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)
- Microplate 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 the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled 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 if 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

Wash Buffer (reagent A.) and Assay Buffer (reagent B.) should be prepared before starting with the test procedure.

A. Wash Buffer

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

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 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 the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of HRP-Conjugate

Just prior to use, predilute the HRP-Conjugate 1:100 by adding 495 µl **Assay Buffer** (reagent B.) to the tube containing the HRP-Conjugate concentrate. Mix the contents of the tube well.

Make a further 1:100 dilution with **Assay Buffer** (reagent B) in a clean plastic tube.

The second dilution (1:100) of the HRP-Conjugate may be prepared as needed according to the following table:

Number of Strips	Prediluted (1:100) HRP-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Please note that the HRP-Conjugate should be used within 30 minutes after predilution. Discard prediluted HRP-Conjugate immediately after use.

D. Preparation of sIL-6R Standard

Reconstitute **sIL-6R Standard** by addition of distilled water. Reconstitution Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard.

Swirl or mix gently to insure complete solubility.

E. Addition of colour-giving reagents: **Blue-Dye**, **Green-Dye**

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (**Blue-Dye**, **Green-Dye**) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the **Blue-Dye** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) according to the test protocol. After addition of **Blue-Dye**, proceed according to the instruction booklet.

5 ml Assay Buffer	20 µl Blue-Dye
12 ml Assay Buffer	48 µl Blue-Dye
50 ml Assay Buffer	200 µl Blue-Dye
60 ml Assay Buffer	240 µl Blue-Dye

2. HRP-Conjugate: Before dilution of the concentrated conjugate, add the **Green-Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of HRP-conjugate.

3 ml Assay Buffer	30 µl Green-Dye
6 ml Assay Buffer	60 µl Green-Dye
12 ml Assay Buffer	120 µl Green-Dye

10. TEST PROTOCOL

- a. Mix all reagents thoroughly without foaming before use.
- b. Predilute serum or plasma samples 1:50 with **Assay Buffer** according to the following dilution scheme:
10 µl Sample + 490 µl **Assay Buffer**
- c. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human sIL-6R from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
- d. Wash the microwell strips twice with approximately 300 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, empty wells and 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 not longer than 15 minutes. Do not allow wells to dry.

- e. Add 100 µl of **Assay Buffer** in duplicate to all standard wells leaving the first wells (5 ng/ml) empty. Prepare standard dilutions by pipetting 200 µl of solubilized (refer to preparation of reagents, 9.D.) **sIL-6R Standard**, in duplicate, into well A1 and A2 (see Figure 1 and 2). Transfer 100 µl to well B1 and B2, respectively. Mix the contents of wells B1 and B2 by repeated aspiration and ejection and transfer 100 µl to well C1 and C2. Take care not to scratch the inner surface of the microwells. Continue this procedure four times, creating two rows of sIL-6R standard dilutions ranging from 5 to 0.08 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of sIL-6R standard dilutions:

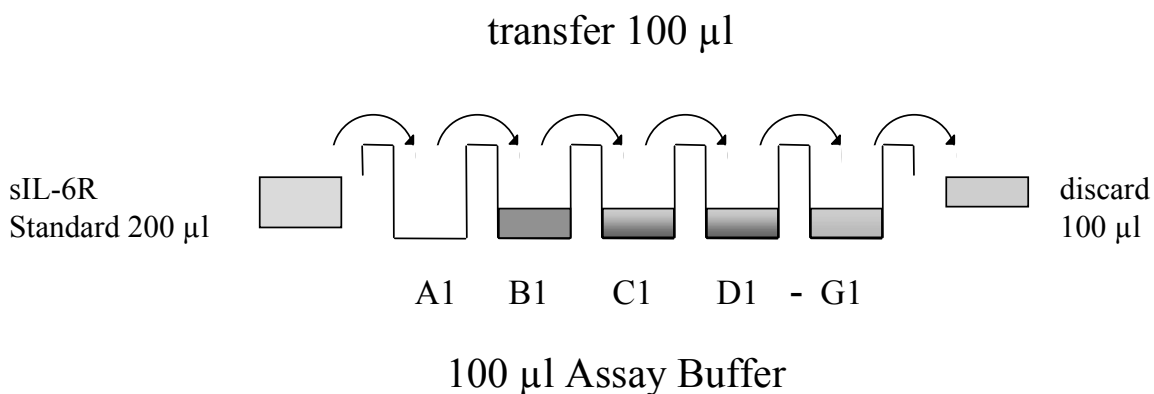


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (5 ng/ml)	Standard 1 (5 ng/ml)	Sample 1	Sample 1
B	Standard 2 (2.5 ng/ml)	Standard 2 (2.5 ng/ml)	Sample 2	Sample 2
C	Standard 3 (1.25 ng/ml)	Standard 3 (1.25 ng/ml)	Sample 3	Sample 3
D	Standard 4 (0.63 ng/ml)	Standard 4 (0.63 ng/ml)	Sample 4	Sample 4
E	Standard 5 (0.31 ng/ml)	Standard 5 (0.31 ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.16 ng/ml)	Standard 6 (0.16 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.08 ng/ml)	Standard 7 (0.08 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- f. Add 100 µl of **Assay Buffer** in duplicate to the blank wells.
- g. Add 80 µl of **Assay Buffer** to all wells designated for samples.
- h. Add 20 µl of each 1:50 prediluted **Sample**, in duplicate, to the designated wells and mix the contents.
- i. Prepare **HRP-Conjugate**. (Refer to preparation of reagents 9.C.)
- j. Add 50 µl of diluted **HRP-Conjugate** to all wells, including the blank wells.
- k. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours, if available on a rotator set at 100 rpm.
- l. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point d. of the test protocol. Proceed immediately to the next step.
- m. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- n. 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 o. of this protocol) before positive wells are no longer properly recordable.

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 an OD of 0.6 – 0.65 is reached.

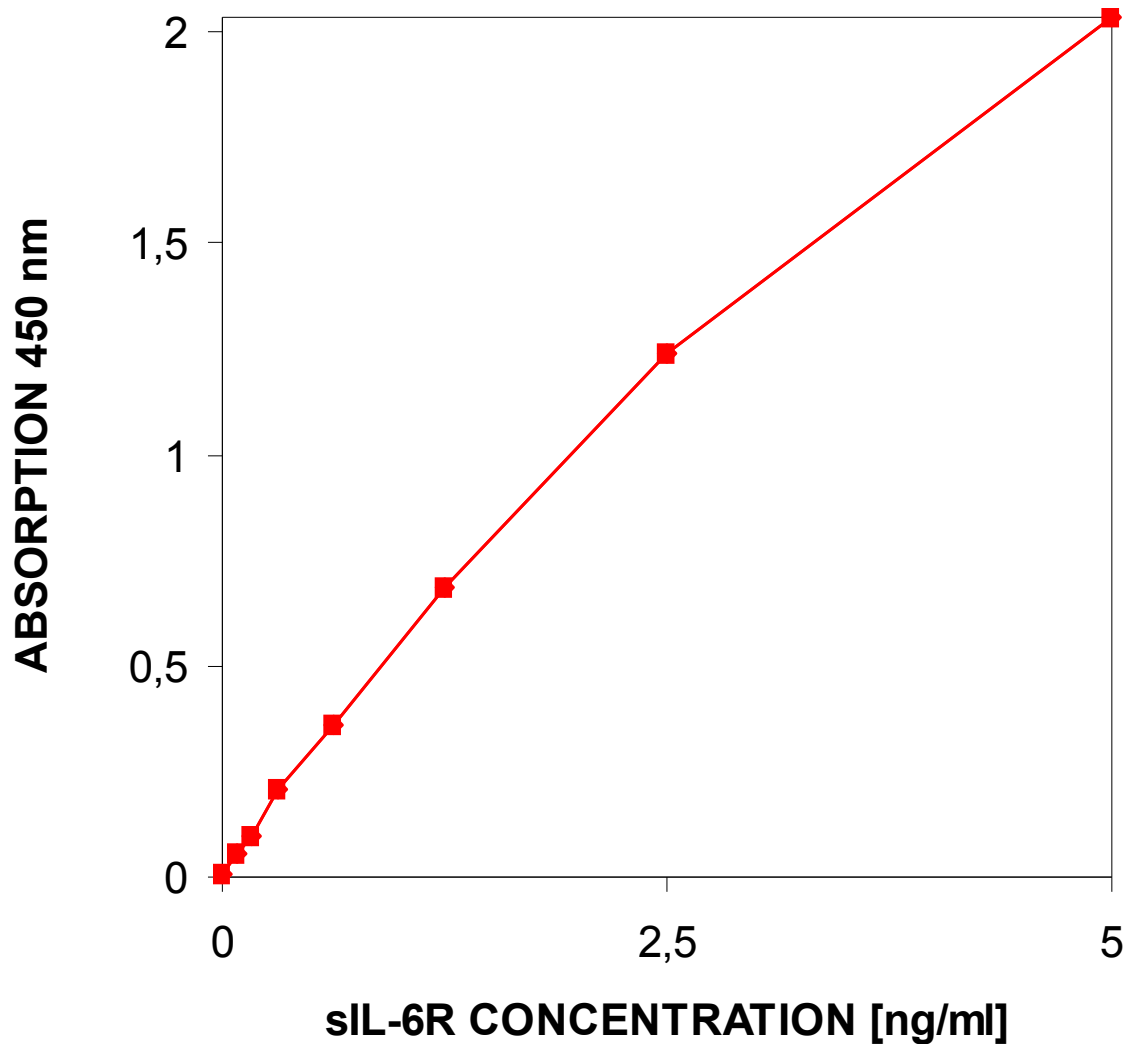
- o. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
- p. 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 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 sIL-6R concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating 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 sIL-6R concentration.
- **For samples which have been diluted according to the instructions given in this manual 1:250, the concentration read from the standard curve must be multiplied by the dilution factor (x 250).**
- **Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sIL-6R levels. Such samples require further dilution of 1:500 - 1:1000 with Assay Buffer in order to precisely quantitate the actual sIL-6R level.**
- It is suggested that each testing facility establishes a control sample of known sIL-6R concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.
- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for sIL-6R ELISA. sIL-6R was diluted in serial two-fold steps in Assay Buffer; symbols represent the mean of three 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.



Typical data using the sIL-6R ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	sIL-6R Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	5	2.072	2.036	2.5
	5	2.000		
2	2.5	1.243	1.237	0.7
	2.5	1.231		
3	1.25	0.696	0.686	2.2
	1.25	0.675		
4	0.63	0.368	0.359	3.5
	0.63	0.350		
5	0.32	0.200	0.205	3.4
	0.32	0.210		
6	0.16	0.105	0.099	8.6
	0.16	0.093		
7	0.08	0.061	0.052	8.2
	0.08	0.046		
Blank	0	0.006	0.007	
	0	0.007		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect 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. Completely empty wells before dispensing fresh Wash Buffer, 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 immunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (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 mouse monoclonal antibodies of irrelevant specificity) are added to the Assay Buffer.

13. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of sIL-6R defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.01 ng/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sIL-6R. Two standard curves were run on each plate. Data below show the mean sIL-6R concentration and the coefficient of variation for each sample. The overall Intra-assay coefficient of variation has been calculated to be 1.7 %.

Positive Sample	Experiment	sIL-6R Concentration (ng/ml)	Coefficient of Variation (%)
1	1	196.6	0.9
	2	192.3	1.8
	3	188.5	1.9
2	1	200.7	0.1
	2	201.6	2.0
	3	196.3	1.6
3	1	169.6	0.4
	2	168.0	0.4
	3	164.4	0.9
4	1	132.8	1.8
	2	137.7	0.7
	3	134.2	2.6
5	1	180.0	1.6
	2	182.8	0.5
	3	177.7	1.5
6	1	137.2	1.0
	2	137.8	4.2
	3	133.4	1.4
7	1	94.7	2.2
	2	105.4	3.3
	3	96.4	4.8
8	1	88.2	0.8
	2	87.8	3.7
	3	90.4	2.1

Negative Sample	O.D. Experiment	Coefficient of 450 nm	Variation (%)
1	1	0.006	38.6
	2	0.006	12.9
	3	0.009	8.3

b. Inter-Assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sIL-6R. Two standard curves were run on each plate. Data below show the mean sIL-6R concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 2.2 %.

Sample	sIL-6R Concentration (ng/ml)	Coefficient of Variation (%)
1	192.4	2.1
2	199.5	1.4
3	167.3	1.6
4	134.9	1.9
5	180.1	1.4
6	136.1	1.8
7	98.8	5.8
8	88.8	1.6

C. Spiking Recovery

Spiked samples were prepared by adding four different levels of recombinant sIL-6R into human serum. As shown below, recoveries were determined in two independent experiments ranging from 94 % to 108 % with an overall mean recovery of 101 %.

sIL-6R Spike (ng/ml)	Experiment	Recovery (%) sIL-6R
4.5	1	97
	2	104
	3	101
2.5	1	94
	2	108
	3	100
1	1	97
	2	101
	3	98
0.5	1	104
	2	106
	3	101

D. Dilution Linearity

Four serum samples with different levels of sIL-6R were assayed at four serial two-fold dilutions (1:250 - 1:2000) with 4 replicates each. In the table below the percent recovery of expected values is listed. Recoveries ranged from 94.6 % to 104.1 % with an overall mean recovery of 99 %.

Sample	Dilution	sIL-6R Concentration (ng/ml)		
		Expected Value	Observed Value	% Recovery of Exp. Value
1	1:250	-	203.2	-
	1:500	101.6	97.4	95.9
	1:1000	50.8	50.0	98.5
	1:2000	25.4	24.0	94.6
2	1:250	-	179.4	-
	1:500	89.7	85.6	95.4
	1:1000	44.8	44.8	99.9
	1:2000	22.4	21.5	95.8
3	1:250	-	205.0	-
	1:500	102.5	99.5	97.1
	1:1000	51.2	53.3	104.1
	1:2000	25.6	26.0	101.6
4	1:250	-	141.1	-
	1:500	70.7	71.1	100.5
	1:1000	35.4	36.0	101.8
	1:2000	17.7	17.2	97.6

E. Expected Values

A panel of 22 sera from apparently healthy blood donors (male and female) was tested for sIL-6R. The detected 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.

F. Sample Freeze-Thaw Stability

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

G. Sample Storage Stability

Aliquots of a serum sample (unspiked or spiked with sIL-6R) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sIL-6R level determined after 24, 48 and 96 hours. There was no significant loss of sIL-6R immunoreactivity during storage under above conditions.

H. 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 sIL-6R determinations. It is nevertheless highly recommended to assure the uniformity of blood preparations.

I. Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a sIL-6R positive serum. There was no detectable cross reactivity. Notably there was no influence on the IL-6R levels measured by the addition of IL-6.

14. REFERENCES

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15. REAGENT PREPARATION SUMMARY

A. Wash Buffer Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer	Number of Strips	Assay Buffer Concentr. (ml)	Distilled Water (ml)
	1 - 6	2.5	47.5
	1 - 12	5.0	95.0

C. HRP-Conjugate Add 495 μ l **Assay Buffer** to tube containing HRP-Conjugate concentrate. Mix. Make further dilution according to table.

	Number of Strips	Prediluted (1:100) HRP-Conjugate (ml)	Assay Buffer (ml)
	1 - 6	0.03	2.97
	1 - 12	0.06	5.94

D. sIL-6R Standard Reconstitute **sIL-6R Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

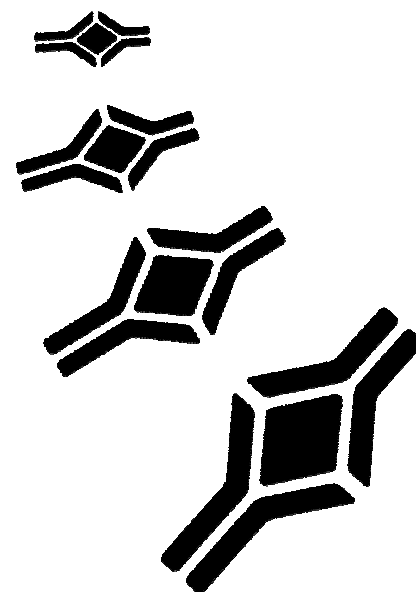
16. TEST PROTOCOL SUMMARY

- Dilute serum or plasma samples with **Assay Buffer** 1:50
- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Assay Buffer**, in duplicate, to standard wells except the first wells (5 ng/ml)
- Pipette 200 µl solubilized **sIL-6R Standard** into the first standard wells and create standard dilutions ranging from 5 to 0.08 ng/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Assay Buffer**, in duplicate, to the blank wells
- Add 80 µl **Assay Buffer** to the sample wells
- Add 20 µl prediluted **Sample**, in duplicate, to designated wells
- Prepare HRP-Conjugate
- Add 50 µl of diluted **HRP-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C)
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 10 to 20 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

Note: For samples which have been diluted according to the instructions given in this manual 1:250, the concentration read from the standard curve must be multiplied by the dilution factor (x 250). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sIL-6R levels. Such samples require further dilution of 1:500 - 1:1000 with **Assay Buffer** in order to precisely quantitate the actual sIL-6R level.

NOTES





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