

Human IL-6 sR ELISA Kit

For the quantitative determination of human interleukin-6 soluble receptor (IL-6 sR) concentrations in serum, plasma, cell culture supernatant, and other biological fluids

Catalogue Number: EL10034

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE



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INTENDED USE

This Human Interleukin-6 soluble receptor ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin-6 soluble receptor (IL-6sR) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH ONLY and is not to be used for diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin-6 (IL-6) is a multifunctional cytokine produced by a wide variety of cell types including monocyte/macrophages, T cells, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astroglomas, and glioblastomas. The effects of IL-6 on different cell types are numerous and varied. These activities include: stimulation of B cell differentiation and antibody secretion; action as a co-stimulant with PHA or Con A to increase IL-2 production and IL-2 receptor expression by T cells; enhancement of differentiation of cytotoxic T cells; action as a growth factor for mature thymic or peripheral T cells, myelomas, hybridomas, plasmacytomas, keratinocytes, and mesangial cells; colony-stimulating activity on hematopoietic cells; induction of neuronal cell differentiation; induction of maturation of megakaryocytes; and stimulation of production of acute phase response proteins by hepatocytes. These various activities indicate that IL-6 plays a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. Elevated IL-6 levels have been reported to be associated with a variety of diseases, including autoimmune diseases, mesangial proliferative glomerulonephritis, psoriasis, and malignancies such as plasmacytomas and myelomas. For reviews of the properties and activities of IL-6, see references 1 to 3.

The biological activities of IL-6 are initiated by binding of the cytokine to a high affinity receptor complex consisting of two membrane glycoproteins; an 80 kDa component receptor that binds IL-6 with low affinity (IL-6R) and a signal-transducing component of 130 kDa (gp130) that does not bind IL-6 by itself, but is required for high affinity binding of IL-6 by the complex. IL-6R and gp130 have been cloned, sequenced and expressed (4-7).

A soluble form of the IL-6 R with a molecular weight of approximately 50 kDa has been found in the urine of healthy adult humans (8), in culture medium conditioned by the growth of a human myeloma cell line (9), in culture supernatants from PHA-stimulated human PBMC and HTLV-1 positive T cell lines (10) and in the serum of HIV-seropositive blood donors (10). This soluble form of the receptor apparently arises from proteolytic cleavage of membrane-bound IL-6R. Soluble forms of human and mouse IL-6 have also been constructed by insertion of termination codons into the regions of the IL-6R cDNAs encoding the external portions of the receptors and prior to the transmembrane domains (11, 12). These soluble receptors have been expressed in COS7 and CHO cells (11, 12) and have been shown to bind IL-6 in solution and to augment the activity of IL-6 as a result of the binding of the IL-6/IL-6 sR complex to membrane-bound gp130 (11, 12). The

regulation *in vivo* of the shedding of the soluble IL-6Rs and the function and significance of these soluble receptors in biological fluids is not currently understood. It has been suggested, however, that pathological states involving elevated levels to IL-6 might also be associated with increased production of soluble IL-6Rs (10).

This IL-6sR ELISA is a 3.5-hour solid phase immunoassay readily applicable to measure IL-6sR levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 2000 pg/mL. It showed no cross reactivity with other cytokines tested. This IL-6sR ELISA is expected to be effectively used for further investigations into the relationship between IL-6sR and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-6 sR enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-6sR. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-6sR and incubated. IL-6sR if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound IL-6sR and other components of the sample. In order to quantitatively determine the amount of IL-6sR present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each have a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3,3',5, 5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-6sR, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm.

In order to measure the concentration of IL-6sR in the samples this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-6sR concentration (pg/mL). The concentration of IL-6sR in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with 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.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

		96 tests
1.	IL-6sR MICROTITER PLATE (Part EL34-1) _____ Pre-coated with anti-human IL-6sR monoclonal antibody.	96 wells
2.	BIOTIN CONJUGATE (Part EL34-2) _____ Anti-human IL-6sR polyclonal antibody conjugated to Biotin.	6 mL
3.	AVIDIN CONJUGATE (Part EL34-3) _____ Avidin conjugated to horseradish peroxidase.	12 mL
4.	IL-6 sR STANDARD (Part EL34-4) _____ Recombinant human IL-6sR (4000 pg/vial) in a buffered protein base with preservative, lyophilized.	2 vials
5.	CALIBRATOR DILUENT I (5X) (EL34-5) _____ Animal serum with buffer and preservative. <i>For serum/plasma testing.</i>	25 mL
6.	CALIBRATOR DILUENT II (EL34-6) _____ Cell culture medium with calf serum and preservative. <i>For cell culture supernatant testing.</i>	25 mL
7.	WASH BUFFER (20X) (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	60 mL
8.	SUBSTRATE A (Part EL34-7) _____ Buffered solution with H ₂ O ₂	10 mL
9.	SUBSTRATE B (Part 30007) _____ Buffered solution with TMB.	10 mL
10.	STOP SOLUTION (Part 30008) _____ 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material!	14 mL

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μ L and 50-200 μ L for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm \pm 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
Solid Waste: Autoclave 60 min. at 121°C.
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING AND STORAGE

- a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
- b) **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
- c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulates.
 - Avoid grossly hemolytic, lipidic or turbid samples.
 - Serum, plasma, and cell culture supernatant samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
 - When performing the assay slowly bring samples to room temperature.
 - It is recommended that all samples be assayed in duplicate.
 - DO NOT USE HEAT-TREATED SPECIMENS.

2. SAMPLE DILUTION

Serum and plasma samples require a final dilution of 1:100 with Calibrator Diluent I (1X). The suggested dilution procedure is as follows:

10µL serum or plasma sample + 990 µL Calibrator Diluent I (1X)

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

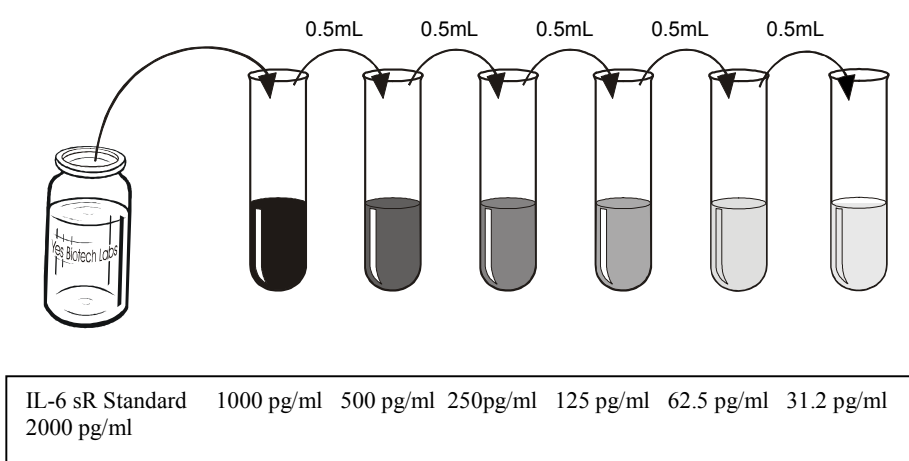
1. **Calibrator Diluent I (1X):** Add 1 volume of Calibrator Diluent I (5X) to 4 volumes of distilled or deionized water. Mix well before use.
2. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

3. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

4. **IL-6 sR Standard:**

- a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-6 sR Standard with 2.0 mL of Calibrator Diluent I (1X) or Calibrator Diluent II. This reconstitution produces a stock solution of 2000 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-6 sR standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.
- b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (31.2 to 2000 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-6 sR stock solution will serve as the high standard (2000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-6sR Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - 0 pg/mL (S1)	2A, 2B	Standard 5 - 250 pg/mL (S6)
1C, 1D	Standard 2– 31.2 pg/mL (S2)	2C, 2D	Standard 6 - 500 pg/mL (S7)
1E, 1F	Standard 3- 62.5 pg/mL (S3)	2E, 2F	Standard 7- 1000 pg/mL (S7)
1G, 1H	Standard 4– 125 pg/mL (S4)	2G, 2H	Standard 8- 2000 pg/mL (S8)
		3A-12H	IL-6 sR samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100µL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.
3. Without discarding the standards and samples, add 50µL Anti-IL-6sR Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.
4. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100 μ L of Avidin Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.
6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
7. Repeat wash procedure as described in Step 4.
8. Add 100 μ L Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
9. Add 100 μ L Stop Solution to each well. Mix well.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-6sR in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-6sR concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL)) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of IL-6sR in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-6sR concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

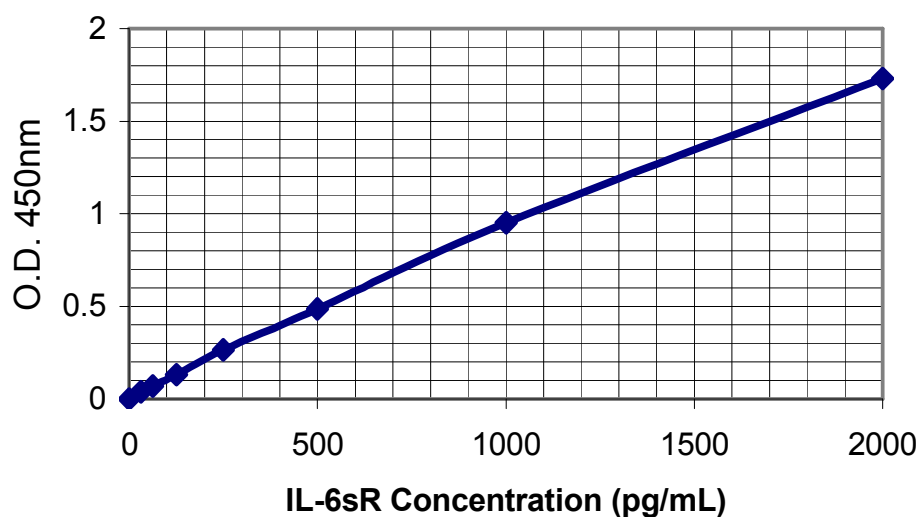
TYPICAL DATA

Results of a typical standard run of an IL-6sR ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE ONE

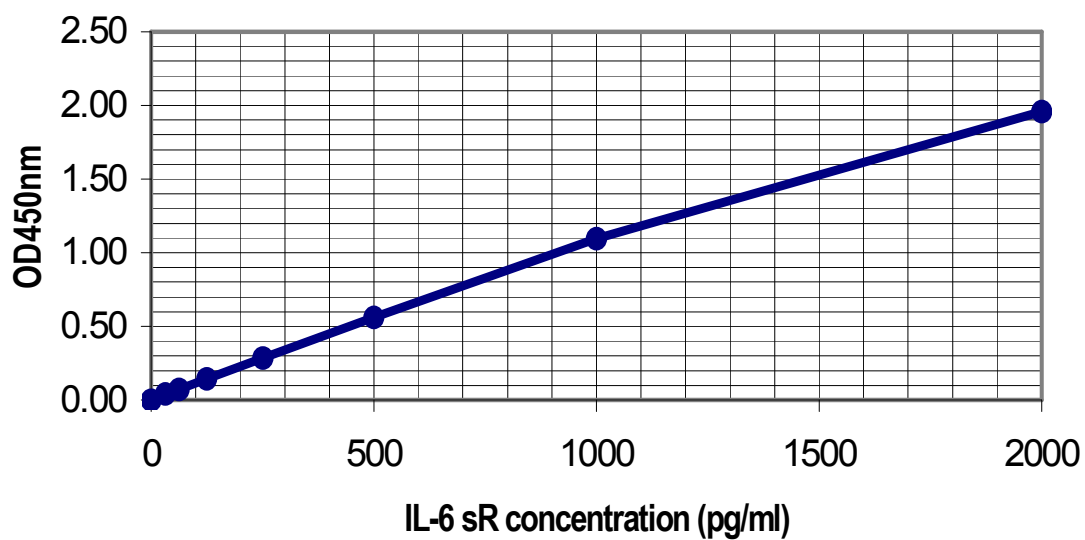
The following data was obtained for a standard curve using Calibrator Diluent I.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.055, 0.059	0.057	0
31.2	0.101, 0.091	0.096	0.039
62.5	0.125, 0.126	0.126	0.069
125	0.191, 0.187	0.189	0.132
250	0.311, 0.335	0.323	0.266
500	0.548, 0.537	0.543	0.486
1000	0.995, 1.020	1.008	0.951
2000	1.803, 1.772	1.788	1.731

**EXAMPLE TWO**

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.062, 0.063	0.063	0
31.2	0.103, 0.107	0.105	0.042
62.5	0.139, 0.132	0.136	0.073
125	0.194, 0.222	0.208	0.145
250	0.351, 0.347	0.349	0.286
500	0.634, 0.611	0.623	0.560
1000	1.151, 1.163	1.157	1.094
2000	2.143, 1.901	2.022	1.959



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by replicates of twenty in 1 assay.

	<i>Calibrator Diluent I Assay</i>		
Sample	1	2	3
n	20	20	20
Mean	235	781	1353
Standard Deviation (pg/ml)	15.7	57.0	96.0
Coefficient of Variation (%)	6.7	7.3	7.1

	<i>Calibrator Diluent II Assay</i>		
Sample	1	2	3
n	20	20	20
Mean (pg/ml)	240	811	1386
Standard Deviation (pg/ml)	16.6	47.0	87.3
Coefficient of Variation (%)	6.9	5.8	6.3

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by replicates on 20 different assays.

	Calibrator Diluent I Assay		
Sample	1	2	3
n	20	20	20
Mean (pg/ml)	232	787	1345
Standard Deviation (pg/ml)	17.4	44.6	86.1
Coefficient of Variation (%)	7.5	5.7	6.4

	Calibrator Diluent II Assay		
Sample	1	2	3
n	20	20	20
Mean (pg/mL)	243	823	1378
Standard Deviation (pg/mL)	17.7	51.0	90.9
Coefficient of Variation (%)	7.3	6.2	6.6

3. RECOVERY

The recovery of IL-6sR spiked to three different levels throughout the range of the assay was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture media	99	82 -105
Serum	101	92 - 109
Plasma	100	85 - 103

4. SENSITIVITY

The minimum detectable dose of IL-6sR was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human IL-6sR using a standard curve generated with Calibrator Diluent I is <7 pg/mL and using Calibrator Diluent II is <7pg/mL.

5. SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human IL-6 sR. This kit exhibits no significant cross-reactivity with factors related to or associated with IL-6 sR such as rhIL-6, rhsgp130 or rmIL-6. No significant cross-reactivity was observed with recombinant human: IL-1 α , IL-1 β , IL-1ra, IL-2 IL-3, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, β -ECGR, EGF, EPO, FGF acidic, FGF basic, FGF-4, G-CSF, GM-CSF, sgp 130, GRO α , IFN- γ , IGF-I IGF-II, LIF, M-CSF, MCP-1, MIP-1 α , MIP-1 β , OSN, PDGF-AA, PDGF-AB, PDGF-BB, RANTES, SCF, SLPI, TGF- α , TGF- β , TNF- α , TNF- β , sTNF RI, sTNF RII.

6. EXPECTED NORMAL VALUES

Biological samples from apparently healthy, normal individuals were collected and the average IL-6 sR concentration measured. Serum samples (n=15) average value: 31,000pg/mL, range: 14,000-46,000pg/mL whereas plasma samples (n=16) average value: 29,000 pg/mL, range: 15,000-48,000 pg/mL.

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