

Human IL-2 sR α ELISA Kit

For the quantitative determination of human interleukin-2 soluble receptor alpha (IL-2 sR α) concentrations in serum, plasma, cell culture supernatant, and other biological fluids.

Catalogue Number: EL10033

96 tests

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

Distributed by Abazyme, LLC

85 Pine Grove St.
Needham, MA, 02494
USA

Tel: 775-363-3329
FAX: 775-206-7905

Email: customer_service@abazyme.com
Web Site: www.abazyme.com

TABLE OF CONTENTS

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
LIMITATION OF THE PROCEDURE	4
REAGENTS PROVIDED	5
MATERIALS REQUIRED BUT NOT SUPPLIED	6
PRECAUTIONS	6
SAMPLE PREPARATION	7
.....Collection, Handling and Storage	7
PREPARATION OF REAGENTS	7
ASSAY PROCEDURE	9
CALCULATION OF RESULTS	11
TYPICAL DATA	11
.....Example one (Calibrator Diluent I)	11
.....Example two (Calibrator Diluent II)	12
PERFORMANCE CHARACTERICS	13
.....Intra-assay precision	13
.....Inter-assay precision	13
.....Recovery	13
.....Sensitivity	14
.....Specificity	14
.....Expected Normal Values	14
REFERENCES	15

INTENDED USE

This Human Interleukin-2 soluble receptor alpha ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin-2 soluble receptor alpha (IL-2 sR α) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not to be used in diagnostic or therapeutic procedures.

INTRODUCTION

The biological activities of IL-2 are mediated by the binding of the factor to a multi-molecular cellular receptor complex. For several years, the receptor was thought to consist of two glycoprotein chains, an alpha chain (IL-2R α) and a beta chain (IL-2R β) (1-3), which acted together to form a high affinity receptor that transduced the IL-2 signal. IL-2 α (also known as Tac antigen and as CD25) is a 55 kDa transmembrane glycoprotein composed of 351 amino acids with only 13 located on the cytoplasmic side of the membrane (4-6). The second chain of the complex was cloned in 1989 (7), and is a transmembrane glycoprotein of 575 amino acids (75kDa), 286 of which are located cytoplasmically and clearly participate in signal transduction (8,9). A third chain, IL-2R γ is necessary for high affinity binding, ligand internalization and signalling. Constitutively expressed on many lymphoid cells, it had been overlooked partly because it has no affinity for IL-2 except when IL-2R β is present (7, 10, 11). When cloned, the gene was found to code for a 64kDa transmembrane protein of 347 amino acids, 84 of which are cytoplasmic (12). Both IL-2R β and IL-2 γ are members of the hematopoietin receptor superfamily, whereas IL-2R α is related only to the IL-15R α chain (13-15).

A model of the IL-2 receptor complex (3, 9, 16-21) would describe the high affinity receptor as an $\alpha\beta\gamma$ trimer, in which all three chains are in contact with the ligand. Alone, IL-2R α binds IL-2 with low affinity, but is unable to transduce a signal. The $\alpha\beta$ combination will bind IL-2 with intermediate affinity, but still will not transduce a signal. A $\beta\gamma$ complex has intermediate affinity and is capable of signalling if the IL-2 concentration is relatively high. Regardless of many subtleties that determine the affinity of the ligand for the extracellular portions of the receptor components (22-24), signalling will ensue if the β and γ cytoplasmic domains are brought into close proximity (25-27).

A soluble form of IL2R α appears in serum, concomitant with its increased expression on cells (18, 28, 29). There are reports of a soluble form of IL-2R β as well (28, 30). The function of the soluble IL-2R α is unclear, since it would be expected to be a poor inhibitor of IL-2 because of its low binding affinity. In any case, increased levels of the soluble IL-2R α in biological fluids reportedly correlate with increased T and B cell activation and immune system activation. Results of a number of studies suggest a correlation of levels of IL-2 sR α in serum with the onset of rejection episodes in allograft recipients (18, 31-33), with activity of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) (34) and with the course of some leukemias and lymphomas (35-40).

This IL-2 sR α ELISA is a 4.5 hour solid phase immunoassay readily applicable to measure IL-2 sR α 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-2 sR α ELISA is expected to be effectively used for further investigations into the relationship between IL-2 sR α and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-2 sR α 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-2 sR α . Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-2 sR α and incubated. IL-2 sR α 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-2 sR α and other components of the sample. In order to quantitatively determine the amount of IL-2sR α 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-2 sR α , 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 450nm \pm 2nm.

In order to measure the concentration of IL-2 sR α 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-2 sR α concentration (pg/mL). The concentration of IL-2 sR α in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR 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 4°C. Refer to the expiration date on the label.

		96 tests
1.	IL-2 sRα MICROTITER PLATE (Part EL33-1) _____ Pre-coated with anti-human IL-2 sR α monoclonal antibody.	96 wells
2.	BIOTIN CONJUGATE (Part EL33-2) _____ Anti-human IL-2 sR α polyclonal antibody conjugated to Biotin.	7 mL
3.	AVIDIN CONJUGATE (Part EL33-3) _____ Avidin conjugated to horseradish peroxidase.	14 mL
4.	IL-2 sRα STANDARD (Part EL33-4) _____ Recombinant human IL-2 sR α (4000 pg/vial) in a buffered protein base with preservative, lyophilized.	2 vials
5.	CALIBRATOR DILUENT I (EL33-5) _____ Animal serum with buffer and preservative. <i>For serum/plasma testing.</i>	22 mL
6.	CALIBRATOR DILUENT II (EL33-6) _____ Cell culture medium with calf serum and preservative. <i>For cell culture supernatant testing.</i>	22 mL
7.	WASH BUFFER (20X) (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	60 mL
8.	SUBSTRATE A (Part 30006) _____ 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

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. *This IL-2 sR α ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.*
 - 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 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.

PREPARATION OF REAGENTS

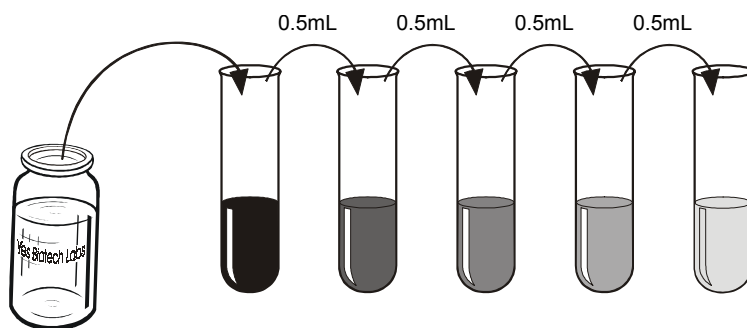
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **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.
2. **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

3. **IL-2 sR α Standard:**

- a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-2 sR α Standard with 2.0 mL of Calibrator Diluent I 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-2 sR α 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 (62.5 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-2 sR α stock solution will serve as the high standard (2000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-2 sR α Standard	2000 pg/ml	1000 pg/ml	500 pg/ml	250pg/ml	125 pg/ml	62.5 pg/ml
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ASSAY PROCEDURE

1. Prepare Wash Buffer and IL-2 sR α 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 and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 0 pg/mL (S1)	2A, 2B	Standard 5 500 pg/mL (S6)
1C, 1D	Standard 2 62.5 pg/mL (S2)	2C, 2D	Standard 6 1000 pg/mL (S7)
1E, 1F	Standard 3 125 pg/mL (S3)	2E, 2F	Standard 7 2000 pg/mL (S7)
1G, 1H	Standard 4 250 pg/mL (S4)	2G, 2H	IL-2 sRα 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 1 drop or 50 μ L of anti-IL-2 sR α biotin conjugate to the antibody pre-coated Microtiter plate.
3. Add 100 μ L of standard or sample to the appropriate well of the antibody pre-coated Microtiter plate. Mix well. Cover and Incubate for 2 hours 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 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add 2 drops or 100 μ L Avidin Conjugate to each well. Cover and incubate for 1 hour at room temperature.
6. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).
7. Repeat wash procedure as described in Step 4.
8. Add 100 μ L Substrate Solution into 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 within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-2 sR α 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-2 sR α 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-2 sR α 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-2 sR α concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

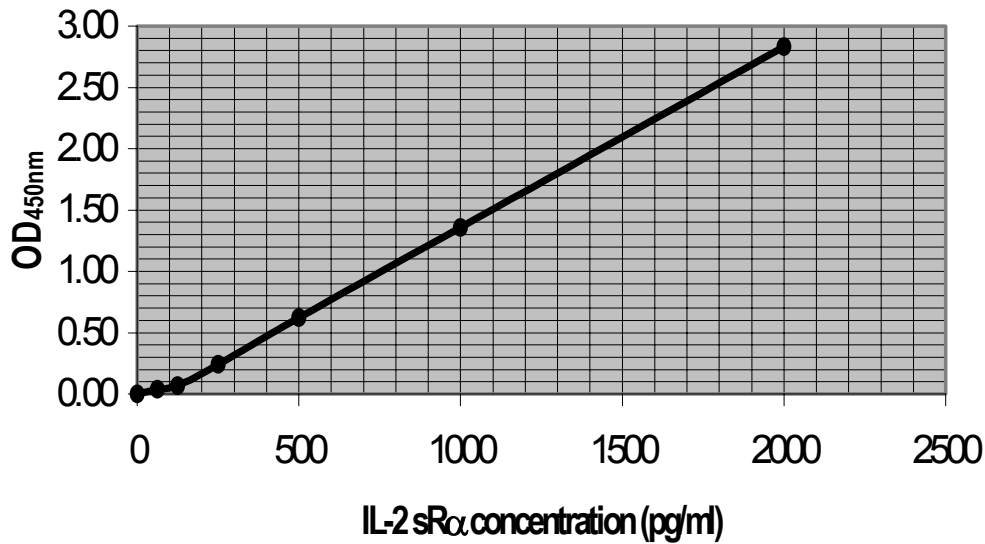
TYPICAL DATA

Results of a typical standard run of an IL-2 sR α 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

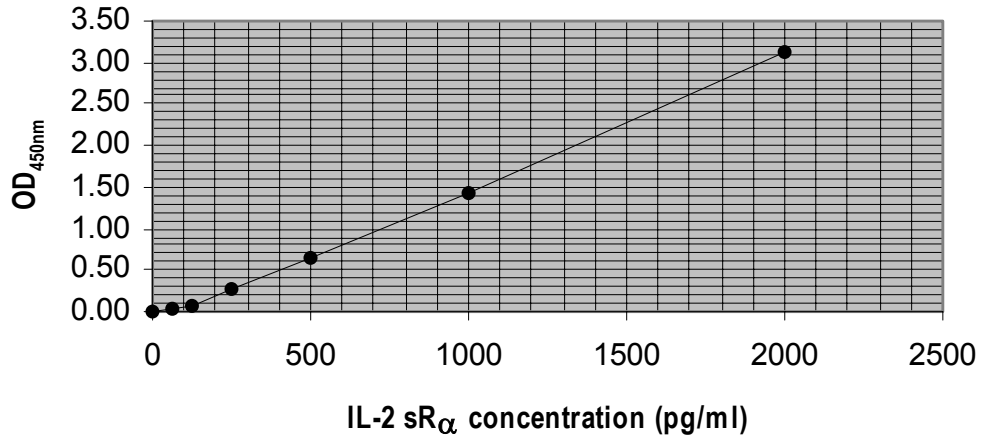
The following data was obtained for a standard curve using Calibrator Diluent I.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.075, 0.063	0.069	0
62.5	0.109, 0.103	0.106	0.037
125	0.124, 0.152	0.138	0.069
250	0.288, 0.336	0.312	0.243
500	0.634, 0.747	0.691	0.621
1000	1.366, 1.484	1.425	1.356
2000	2.841, 2.955	2.898	2.829

**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	0.102, 0.094	0.098	0
62.5	0.132, 0.122	0.127	0.029
125	0.178, 0.176	0.177	0.079
250	0.390, 0.349	0.369	0.271
500	0.755, 0.729	0.742	0.644
1000	1.522, 1.504	1.513	1.415
2000	3.146, 3.315	3.231	3.133



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 (pg/ml)	158	426	431
Standard Deviation (pg/ml)	10.5	26.3	35.9
Coefficient of Variation (%)	6.6	6.1	8.3

	<i>Calibrator Diluent II Assay</i>		
Sample	1	2	3
n	20	20	20
Mean (pg/ml)	164	443	952
Standard Deviation (pg/ml)	9.8	29.5	39.2
Coefficient of Variation (%)	5.9	6.6	4.1

2. INTER-ASSAY PRECISION

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

	<i>Calibrator Diluent I Assay</i>		
Sample	1	2	3
n	20	20	20
Mean (pg/ml)	160	451	925
Standard Deviation (pg/ml)	11.5	24.6	41.5
Coefficient of Variation (%)	7.1	5.5	4.5

	<i>Calibrator Diluent II Assay</i>		
Sample	1	2	3
n	20	20	20
Mean (pg/mL)	142	436	971
Standard Deviation (pg/ml)	9.8	24.2	39.9
Coefficient of Variation (%)	6.9	5.6	4.1

3. RECOVERY

The recovery of IL-2 sR α spiked to seven different levels in five test samples throughout the range of the assay was evaluated. All samples were mixed and assayed in duplicate

Sample Type	Average Recovery (%)	Range (%)
Cell Culture Media	104	95-115
Serum	105	100-118
EDTA plasma	104	93-110
Heparin plasma	104	95-111
Citrate plasma	105	92-116

4. SENSITIVITY

The minimum detectable dose of IL-2 sR α 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-2 sR α using a standard curve generated with Calibrator Diluent I is <10 pg/mL and using Calibrator Diluent II is <10 pg/mL.

5. SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human IL-2 sR α . This kit exhibits no significant cross-reactivity with factors related to or associated with IL-2 sR α such as rhIL-2, rhIL-2 sR α . No significant cross-reactivity was observed with recombinant human: IL-1 α , IL-1 β , IL-1 sRI, IL-1 sRII, IL-1ra, IL-3, IL-3 sR α , IL-5, IL-5 sR α , IL-5 sRb, IL-6, IL-6 sR, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13 ANG, AR, CNTF, β -ECGR, EGF, EPO, FGF acidic, FGF basic, FGF-4, FGF-5, FGF-6, FGF-7, G-CSF, GM-CSF, sgp 130, GRO α , GRO β , GRO γ , HB-EGF, HGF, INF- γ , IGF-I IGF-II, LAP (TGF- β 1), LIF, M-CSF, MCP-1, MIP-1 α , MIP-1 β , β -NGF, OSN, PD-ECGF, PDGF-AA, PDGF-AB, PDGF-BB, PTN, RANTES, SCF, SLPI, TGF- α , TGF- β , TNF- α , TNF- β , sTNF RI, sTNF RII, VEGF.

6. EXPECTED NORMAL VALUES

Biological samples from apparently healthy, normal individuals were collected and the average IL-2 sR α concentration measured. Serum samples (n=15) average value: 1265 pg/ml, range: 650-2216 pg/mL whereas plasma samples (n=15) average value: 1127 pg/ml, range: 540-3150 pg/mL.

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