

# Human IL-11 ELISA Kit

For the quantitative determination of human Interleukin-11 (IL-11) concentrations in serum, plasma, and cell culture supernatant.

Catalogue Number: EL10042

*96 tests*

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

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## **INTENDED USE**

This Human IL-11 ELISA kit is to be used for the *in vitro* quantitative determination of human Interleukin 11 (IL-11) concentrations in serum, plasma, and cell culture supernatant. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

## **INTRODUCTION**

Interleukin 11 (IL-11) was originally discovered as a factor produced by an IL-1-stimulated bone marrow stromal cell line that was mitogenic for an IL-6-responsive mouse plasmacytoma cell line (16). The human IL-11 cDNA encodes a 199 amino acid residue precursor polypeptide with a 21 amino acid residue hydrophobic signal that is processed proteolytically to generate the 178 amino acid residue mature IL-11. IL-11 has no cysteine residues or potential glycosylation sites. It is highly helical and thermally stable (4). The amino acid sequences of human and primate IL-11 molecules are 94% identical; human and mouse molecules are 88% identical (6). IL-11 is produced by a variety of mesenchymal cells (7), with expression either transcriptionally or post-transcriptionally regulated (6-8), depending on the cell. It exerts biological activity through a specific receptor, IL-11R (3,11), that signals through gp130 (9,11), the signalling subunit for IL-6, LIF, OSM, and CNTF. IL-11 binds to the IL-11R protein alone with low affinity; the affinity is high when IL-11R is associated with gp130 (11). IL-11R mRNA has been reported in a wide variety of cells and tissues (4).

IL-11 is synergistic with IL-3, IL-4, IL-7, IL-12, IL-13, SCF, Flt-3 ligand and GM-CSF in stimulating proliferation of hematopoietic progenitors (4,13-15). It stimulates megakaryocytopoiesis and thrombopoiesis in synergism with IL-3, thrombopoietin and SCF. It stimulates erythropoiesis, myelopoiesis and lymphopoiesis, and it modulates the hematopoietic microenvironment (4). Alveolar and bronchial epithelial cells produce IL-11 in response to inflammatory cytokines (8), and IL-11 modulates growth of GI epithelial cells (2). IL-11 stimulates osteoclast development (10), it stimulates acute-phase reactants (1) and it inhibits adipogenesis (12). IL-11 activity can be measured in a cell proliferation assay based on its ability to support the growth of a sub-line of the mouse plasmacytoma cell line (T1165.85.2.1) that has been adapted to grow in IL-11 (16). The bioassay is time-consuming and not completely specific for IL-11.

The IL-11 Immunoassay is a 3.5 hour, solid-phase ELISA designed to measure IL-11 in cell culture supernatant, serum, or plasma. It contains recombinant human IL-11 and antibodies raised against recombinant human IL-11. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-11 showed dose curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that the kit can be used to determine relative mass values for natural IL-11.

## **PRINCIPLE OF THE ASSAY**

This IL-11 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-11. Standards or samples are then added to the appropriate microtiter plate wells and incubated. After washing to remove unbound IL-11 and other components of the sample, biotin-conjugated polyclonal antibody specific to IL-11 is added and incubated. IL-11, if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by the biotin conjugate. In order to quantitatively determine the amount of IL-11 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 has 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-11, 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 ± 2 nm.

In order to measure the concentration of IL-11 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 standard provided 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-11 concentration (pg/mL). The concentration of IL-11 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 4°C. Refer to the expiration date on the label.

		<b>96 tests</b>
1.	<b>IL-11 MICROTITER PLATE</b> (Part 42009) _____ Pre-coated with murine anti-human IL-11 monoclonal antibody.	<b>96 wells</b>
2.	<b>BIOTIN CONJUGATE</b> (Part 42010) _____ Anti-human IL-11 polyclonal antibody conjugated to Biotin.	<b>11 mL</b>
3.	<b>AVIDIN CONJUGATE</b> (Part 42011) _____ Avidin conjugated to horseradish peroxidase.	<b>14 mL</b>
4.	<b>IL-11 STANDARD</b> (Part 42012) _____ Recombinant human IL-11 (2000 pg/vial) in a buffered protein base with preservative, lyophilized.	<b>2 vials</b>
5.	<b>CALIBRATOR DILUENT I</b> (Part 30003) _____ Animal protein with buffer and preservative. <i>For serum/plasma testing.</i>	<b>22 mL</b>
6.	<b>CALIBRATOR DILUENT II</b> (Part 30004) _____ Cell culture medium with animal protein and preservative. <i>For cell culture supernatant testing.</i>	<b>22 mL</b>
7.	<b>WASH BUFFER (20X)</b> (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	<b>60 mL</b>
8.	<b>SUBSTRATE A</b> (Part 30006) _____ Buffered solution with H <sub>2</sub> O <sub>2</sub>	<b>10 mL</b>
9.	<b>SUBSTRATE B</b> (Part 30007) _____ Buffered solution with TMB.	<b>10 mL</b>
10.	<b>STOP SOLUTION</b> (Part 30008) _____ 2N Sulphuric Acid (H <sub>2</sub> SO <sub>4</sub> ). Caution: Caustic Material!	<b>14 mL</b>

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 $\mu$ L and 50-200 $\mu$ 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 Wastes: Autoclave for 60 minutes at 121°C.  
Liquid Wastes: 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 virus 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:** Collect cell culture supernatant, Centrifuge to remove any visible pellets. Assay can be immediately conducted or samples can be aliquoted and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- b) **Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature. Centrifuge for 10 minutes at 1000 x g ( $4^{\circ}\text{C}$ ). Remove serum and assay (see activation procedure) immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- c) **Plasma:** Collect plasma on ice using EDTA as an anticoagulant. Centrifuge at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 30 minutes at  $2-8^{\circ}\text{C}$  is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

## PREPARATION OF REAGENTS

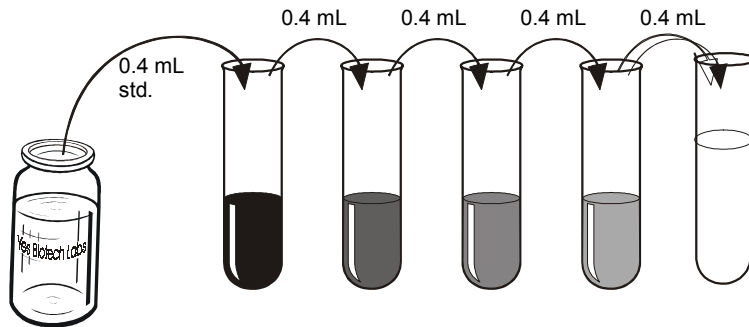
Remove all kit reagents from refrigerator and allow them to reach room temperature ( $20-25^{\circ}\text{C}$ ). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

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  $2-8^{\circ}\text{C}$ . 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 below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
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-11 Standard:**

- a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-11 Standard with either 1.25 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 1600 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-11 standard stock solution must be stored frozen (-20°C) immediately after use so that it can last 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 within the range of this assay (0 pg/mL to 1600 pg/mL) as illustrated. Add 0.4 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-11 Standard will serve as the **high standard (1600 pg/mL)** and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-11 Standard 1600 pg/mL	800 pg/mL	400 pg/mL	200 pg/mL	100 pg/mL	50 pg/mL
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## ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-11 Standards and activated sample before starting assay procedure. *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
<b>1A, 1B</b>	Standard 1 - 0 <b>pg/mL</b> (S1)	<b>2A, 2B</b>	Standard5 - 400 <b>pg/mL</b> (S5)
<b>1C, 1D</b>	Standard 2 - 50 <b>pg/mL</b> (S2)	<b>2C, 2D</b>	Standard6 - 800 <b>pg/mL</b> (S6)
<b>1E, 1F</b>	Standard 3 - 100 <b>pg/mL</b> (S3)	<b>2E, 2F</b>	Standard7- 1600 <b>pg/mL</b> (S7)
<b>1G, 1H</b>	Standard4 - 200 <b>pg/mL</b> (S4)	<b>2G, 12H</b>	IL-11 samples

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

2. Add 100  $\mu$ L of Standard or activated sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.
3. 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 one more time for a **total of two 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 **two times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350  $\mu$ L/well/wash (range: 350-400  $\mu$ L). 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.*

4. Dispense two (2) drops or 100  $\mu$ L biotin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

5. Repeat wash procedure as described in Step 3. Wash plate **five times**.
6. Dispense two (2) drops or 100  $\mu\text{L}$  avidin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.
7. Repeat wash procedure as described in Step 5.
8. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
9. Add 100  $\mu\text{L}$  Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
10. Add 100  $\mu\text{L}$  Stop Solution to each well. Mix well.
11. 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-11 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-11 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) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of IL-11 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-11 concentration.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay. A suggested 10-fold dilution is 50  $\mu\text{L}$  sample + 450  $\mu\text{L}$  Calibrator Diluent I.

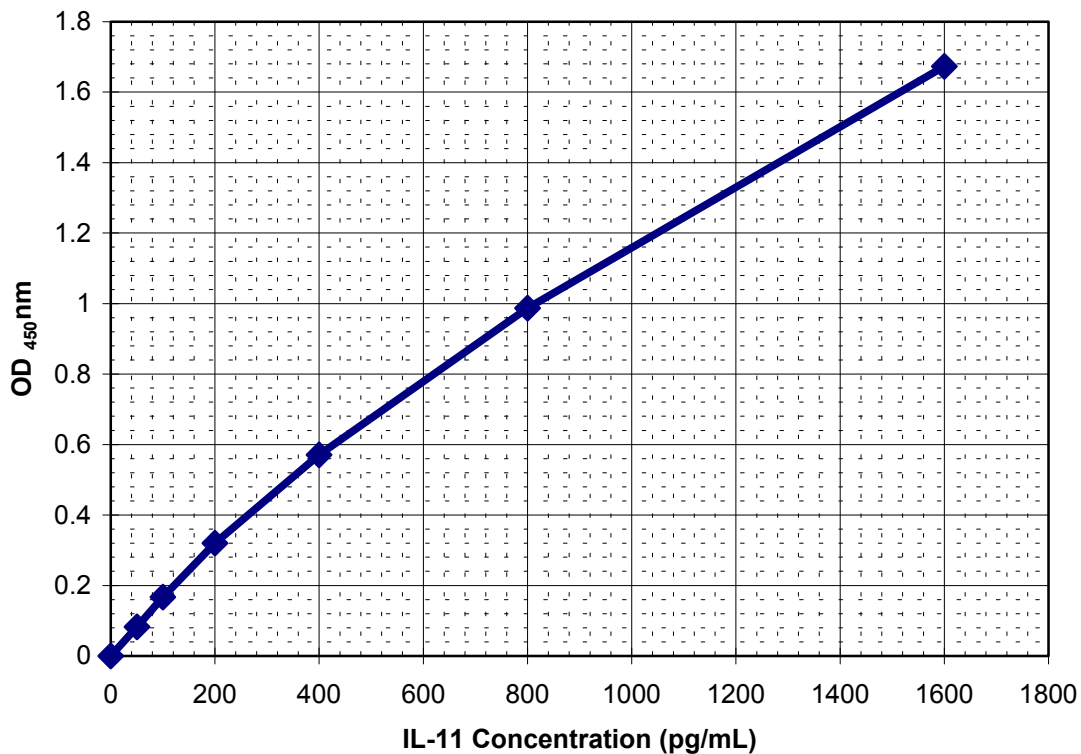
## TYPICAL DATA

Results of a typical standard run of a IL-11 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.

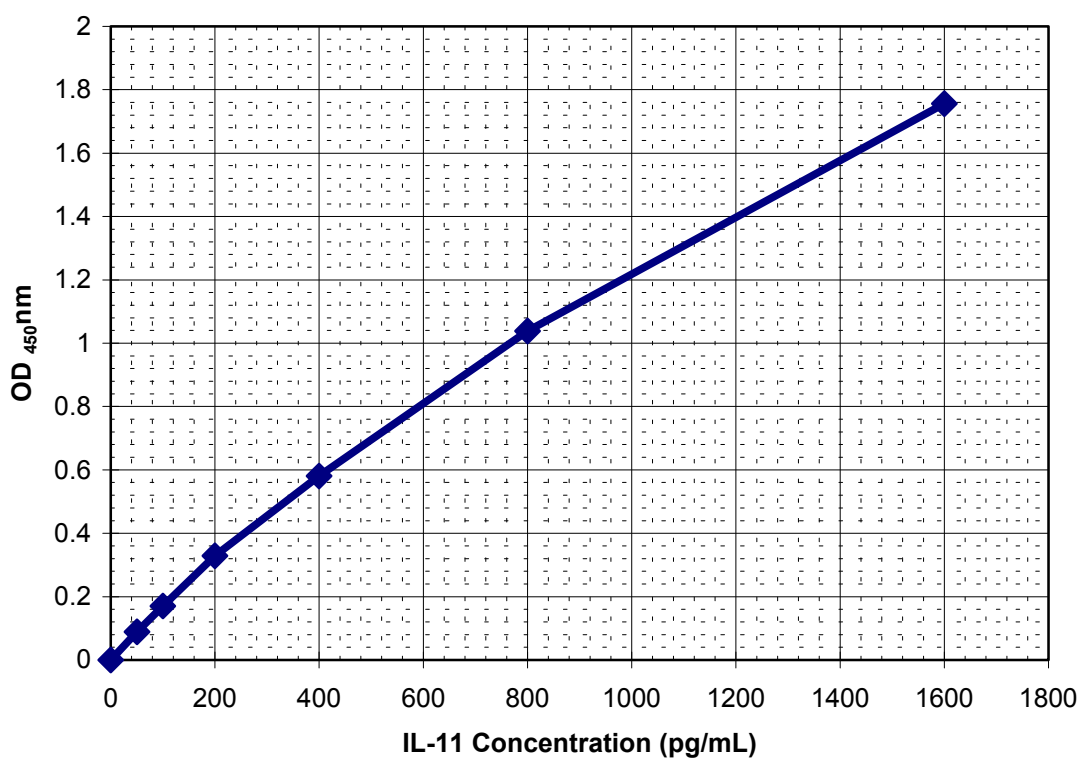
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.061, 0.057	0.059	0
50	0.145, 0.139	0.142	0.083
100	0.228, 0.223	0.226	0.167
200	0.385, 0.372	0.379	0.320
400	0.641, 0.618	0.630	0.571
800	1.057, 1.035	1.046	0.987
1600	1.714, 1.750	1.732	1.673



## 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	0.063, 0.065	0.064	0
50	0.150, 0.156	0.153	0.089
100	0.230, 0.237	0.234	0.170
200	0.395, 0.391	0.393	0.329
400	0.635, 0.654	0.645	0.581
800	1.100, 1.103	1.102	1.038
1600	1.818, 1.821	1.820	1.756



## PERFORMANCE CHARACTERISTICS (Quantikine)

### 1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

Sample	Calibrator Diluent I assay			Calibrator Diluent II assay		
	1	2	3	1	2	3
n	16	16	16	16	16	16
Mean (pg/mL)	50	300	1000	50	300	1000
Standard Deviation (pg/mL)	2.0	15.1	50.7	1.8	14.5	48.0
Coefficient of Variation (%)	4.0	5.0	5.1	3.6	4.8	4.8

### 2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 16 different assays.

Sample	Calibrator Diluent I assay			Calibrator Diluent II assay		
	1	2	3	1	2	3
n	16	16	16	16	16	16
Mean (pg/mL)	50	300	1000	50	300	1000
Standard Deviation (pg/mL)	3.9	20.1	60.9	3.8	18.9	70.0
Coefficient of Variation (%)	7.8	6.7	6.1	7.6	6.3	7.0

### 3. RECOVERY

The recovery of IL-11 spiked to levels throughout the range of the assay followed by activation in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	98	90-106%
Serum	97	88-120%
EDTA plasma (platelet-poor)	108	94-123%

### 4. SENSITIVITY

The minimum detectable dose of IL-11 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is 4.0 pg/mL and using Calibrator Diluent II is 3.0 pg/mL.

## 5. SPECIFICITY

This sandwich ELISA recognizes both natural and recombinant human IL-11. The factors listed below were prepared at 50 ng/mL. In Calibrator Diluent I and Calibrator Diluent II and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL. In a mid-range rhIL-11Control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human				Recombinant Mouse	
IL-1 $\alpha$	ANG	IGF-I	SLP1	IL-1 $\alpha$	bFGF acidic
IL-1 $\beta$	CNTF	LIF	TNF- $\alpha$	IL-1 $\beta$	bFGF basic
IL-1 ra	$\beta$ -ECGF	M-CSF	TNF- $\beta$	IL-3	mEGF
IL-2	EGF	MCP-1	sTNF RI	IL-4	
IL-3	EPO	MIC-1 $\alpha$	sTNF RII	IL-5	
IL-4	FGF-basic	MIP-1 $\beta$	VEGF	IL-7	
IL-5	FGF-acidic	$\beta$ -NGF		IL-9	
IL-6	FGF-5	OSM		IL-10	
IL-6 sR	FGF-6	PDGF-AA		EGF	
IL-7	G-CSF	PDGF-AB		GM-CSF	
IL-8	GRO- $\alpha$	PDGF-BB		LIF	
IL-9	HB-EGF	PTN		MIP-1 $\beta$	
IL-10	HGF	PANTES		SCF	
IL-11	IFN- $\gamma$	SCF		TNF- $\alpha$	

## 6. CALIBRATION

This immunoassay is calibrated against NIBSC Standard (Reference preparation) Code No. 92/788.

## 7. SAMPLE VALUES

**Serum/Plasma** - Forty serum, EDTA plasma, heparin and citrate plasma samples were evaluated for the presence of IL-11 in this assay. All samples measured less than the lowest IL-11 standard, 50 pg/mL.

**Cell Culture Supernate** - MRC-5 cells ( $1 \times 10^6$  cells/mL) were cultured in DME supplemented with 10% FBS plus 2 mM L-glutamine, 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin sulfate, 10 ng/mL PMA and 1 ng/mL IL-1 $\alpha$ . Aliquots of the culture supernate were removed on day 2 and assayed for levels of natural IL-11.

Condition	Day 2 (pg/mL)
Unstimulated	2,400
Stimulated	10,000

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