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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S7.5 (05) IL-11

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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, also known as adipogenesis inhibitory factor (AGIF) and oprelvekin, is a member of the GP130 family of cytokines. These cytokines drive the assembly of multi-subunit receptor complexes containing at least one GP130. IL-11 exerts biological activity through IL-11 receptor by utilizing the gp130 molecule as the signaling component of its receptor. Interleukin 11 (IL-11) is a highly conserved precursor protein of 19kDa with 199 amino acids. The first 21 amino acids are a typical leader sequence for secreted proteins. When the 178 amino acid protein is released from the cell, the first 21 amino acids are removed. IL-11 is thermally stable and contains no cysteine residues. Many cell and tissue types throughout the body, such as CNS, thymus, lung, bone, connective tissues, uterus, skin, and testis, can produce IL-11 depending on the local environment of the cell or tissue. IL11 promotes primary and secondary immune responses *in vitro* and *in vivo* and modulates antigen-specific antibody reactions. IL-11 has activity in bone metabolism and activity in protection and restoration of the gastrointestinal mucosa. Human endometrial stromal cells produce biologically active IL-11, which promotes progesterone-induced decidualization. Therefore, the IL-11 has both paracrine and autocrine actions on human endometrial stromal cells and plays an important role in preparing the human endometrium for implantation. Reports indicate that abnormal IL-11 levels in serum or plasma is related to immune thrombocytopenia, rheumatoid arthritis, and urogenital infection. Immunoreactive IL-11 has also been linked to aseptic loosening of total hip replacement implants. The production of IL-11 is elevated in lesional skin organ cultures of patients with active plaque-type psoriasis as compared with non-lesional and normal skin.

The human IL-11 genes contain five exons, each having a length of 7 kDa, and are mapped to chromosome 19q13. 3-13. 4. Primate and human IL-11 sequences show 97 percent homology. The human IL-11 receptor gene maps to chromosome 9p13.

This IL-11 ELISA Kit is a ready-to-use 3.5-hour solid phase immunoassay capable of measuring IL-11 levels in serum, plasma, cell culture supernatant in the range of 0 to 1600 pg/mL. This kit has been shown to have a specific reaction with IL-11 without any cross reactivity with various other cytokine super-family proteins.

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. If present, IL-11 will bind and become immobilized by the antibody pre-coated on the wells and then become “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, each having 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 APPLICATION

- The Human IL-11 ELISA kit is for laboratory research use only, and is not intended for use in clinical diagnostic procedures.
- Although all manufacturing precautions have been exercised to ensure that this product will be suitable for use with all validated sample types as designated in the product insert, the possibility of interference cannot be excluded due to the variety of proteins that may exist within the sample.
- The Calibrator Diluent selected for the standard curve should be consistent with the assay samples. If the values generated by the samples are greater than the uppermost standard, the samples dilution should be adjusted with the appropriate Calibrator Diluent and the assay should be repeated.

REAGENTS PROVIDED

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

	96 tests

1. **IL-11 MICROTITER PLATE** (Part EL42-1) ***96 wells***
   Pre-coated with murine anti-human IL-11 monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL42-2) ***6 mL***
   Anti-human IL-11 polyclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL42-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-11 STANDARD** (Part EL42-4) ***2 vials***
   Recombinant human IL-11 (2000 pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL42-5) ***25 mL***
   Animal protein with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL42-6) ***25 mL***
   Cell culture medium with animal protein and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL42-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL 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 ± 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 ≤-20°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°C). Remove serum and assay (see activation procedure) immediately or aliquot and store at ≤-20°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°C is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

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.

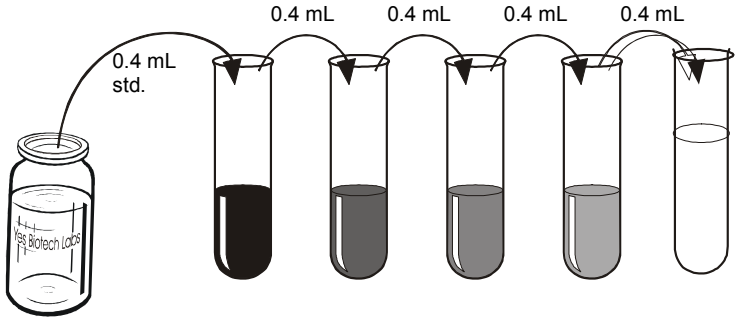
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

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. **<u>IL-11 Standard</u>:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the IL-11Standard 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 <u>appropriate</u> <u>Calibrator</u> <u>Diluent</u> 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

ASSAY PROCEDURE

1. Prepare Wash Buffer and IL-11 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	**400 pg/mL**	(S5)
1C, 1D	Standard 2	**50 pg/mL**	(S2)	**2C, 2D**	Standard 6	**800 pg/mL**	(S6)
1E, 1F	Standard 3	**100 pg/mL**	(S3)	**2E, 2F**	Standard 7	**1600 pg/mL**	(S7)
1G, 1H	Standard4	**200 pg/mL**	(S4)	**2G,2H**	**IL-11** 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 IL-11 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 100µl 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 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100µL 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-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 µL sample + 450 µL Calibrator Diluent I.

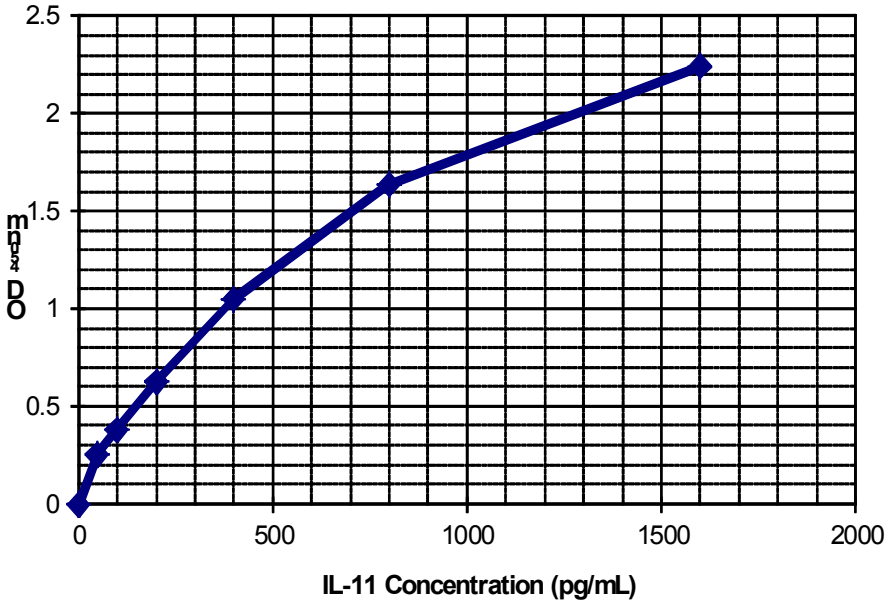
TYPICAL DATA

Results of a typical standard run of an 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, 0.059	0.06	0.000
50	0.257, 0.245	0.251	0.191
100	0.375, 0.391	0.383	0.323
200	0.618, 0.638	0.628	0.568
400	1.035, 1.067	1.051	0.991
800	1.642, 1.629	1.635	1.576
1600	2.240, 2.234	2.237	2.177



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

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

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**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. I**NTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by using replicates on 16 different assays.

	Calibrator Diluent I assay			*Calibrator Diluent II assay*		
Sample	**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. R**ECOVERY**
   By employing random samples, the recovery of IL-11 was evaluated at various amounts of IL-11 throughout the range of the assay.

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

4. S**ENSITIVITY**
   The minimum detectable quantities of human IL-10 as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 4.0 pg/mL and 3.0pg/mL respectively. The two standard deviations above the mean optical density of the 16 replicates of the zero standard were defined as the minimum detectable quantities.

5. S**PECIFICITY**
   This sandwich ELISA can detect both natural and recombinant human IL-11. The following factors were assayed for cross-reactivity at 50 ng/mL in Calibrator Diluent I and Calibrator Diluent II. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-11Control were tested for interference. No significant cross-reactivity or interference was identified.

Recombinant Human				Recombinant Mouse	
IL-1α	ANG	IGF-I	SLP1	IL-1α	bFGF acidic
IL-1β	CNTF	LIF	TNF-α	IL-1β	bFGF basic
IL-1 ra	β-ECGF	M-CSF	TNF-β	IL-3	mEGF
IL-2	EGF	MCP-1	sTNF RI	IL-4	
IL-3	EPO	MIC-1α	sTNF RII	IL-5	
IL-4	FGF-basic	MIP-1β	VEGF	IL-7	
IL-5	FGF-acidic	β-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-α	PDGF-BB		LIF	
IL-9	HB-EGF	PTN		MIP-1β	
IL-10	HGF	PANTES		SCF	
IL-11	IFN-γ	SCF		TNF-α	

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

7. **EXPECTED NORMAL VALUES**

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

   Cell Culture Supernatant - MRC-5 cells (1 x 10^6 cells/mL) were cultured in DME supplemented with 10% FBS plus 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, 10 ng/mL PMA and 1 ng/mL IL-1α. Aliquot the culture supernatant and assay on Day 2 to measure the quantity of natural IL-11.

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

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