# Quantikine® ELISA

## Human IL-11 Immunoassay

Catalog Number D1100

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

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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# 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 (1). For reviews of IL-11 see references 2 and 3. The human IL-11 cDNA encodes a 199 amino acid (aa) residue precursor polypeptide with a 21 aa residue hydrophobic signal that is processed proteolytically to generate the 178 aa residue mature IL-11. IL-11 has no cysteine residues or potential glycosylation sites. It is highly helical and thermally stable (4). The aa sequences of human and primate IL-11 molecules are 94% identical; human and mouse molecules are 88% identical (3).

IL-11 is produced by a variety of mesenchymal cells (5), with expression either transcriptionally or post-transcriptionally regulated (6 - 8), depending on the cell. It exerts biological activity through a specific receptor, IL-11 R (9, 10), that signals through gp130 (9, 11), the signalling subunit for IL-6, LIF, OSM, and CNTF. IL-11 binds to the IL-11 R protein alone with low affinity; the affinity is high when IL-11 R is associated with gp130 (9). IL-11 R mRNA has been reported in a wide variety of cells and tissues (4).

IL-11 is synergistic with IL-3, -4, -7, -12, -13, SCF, Flt-3 ligand and GM-CSF in stimulating proliferation of hematopoietic progenitors (4, 12 - 14). 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 (15), and IL-11 modulates growth of GI epithelial cells (16). IL-11 stimulates osteoclast development (17), stimulates acute-phase reactants (18) and inhibits adipogenesis (19).

The Quantikine® Human IL-11 Immunoassay is a 4.5-5.0 hour, solid-phase ELISA designed to measure human IL-11 in cell culture supernates, serum, or plasma. It contains *Sf* 21-expressed 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 Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-11.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-11 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-11 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-11 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-11 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- 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 diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Human IL-11 Microplate** | 890202 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-11. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Human IL-11 Standard** | 890204 | 2 vials of recombinant human IL-11 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Use a fresh standard for each assay. Discard the IL-11 stock solution and dilutions after 4 hours. |
| **Human IL-11 Conjugate** | 890203 | 21 mL of a polyclonal antibody specific for human IL-11 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1S** | 895137 | 11 mL of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5K** | 895119 | 21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.* | |
| **Calibrator Diluent RD6N** | 895135 | 21 mL of animal serum with preservatives. *For serum/plasma samples.* | |
| **Wash Buffer Concentrate** | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 12 mL of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 6 mL of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 adhesive strips. | |

* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human IL-11 Controls (optional; R&D Systems®, Catalog # QC21).

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Cell culture supernate samples may require a dilution prior to assay. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5K.

## REAGENT PREPARATION

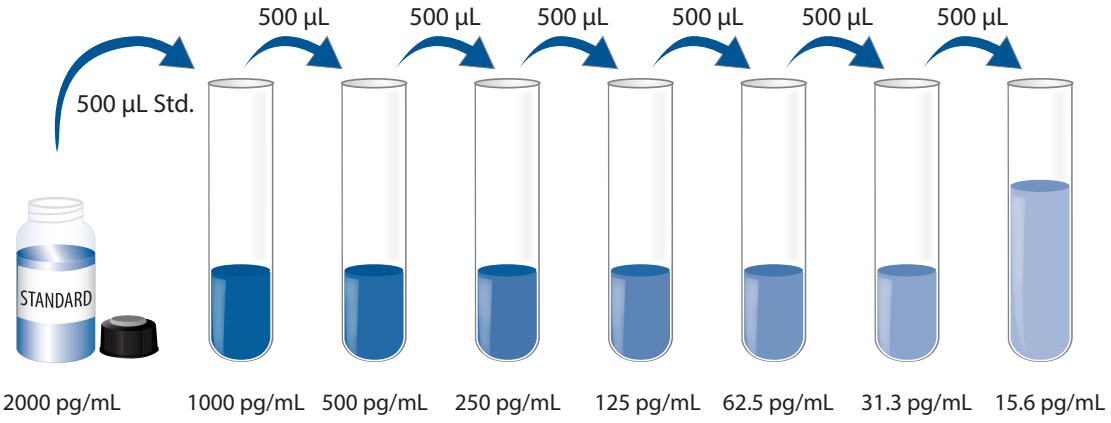
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

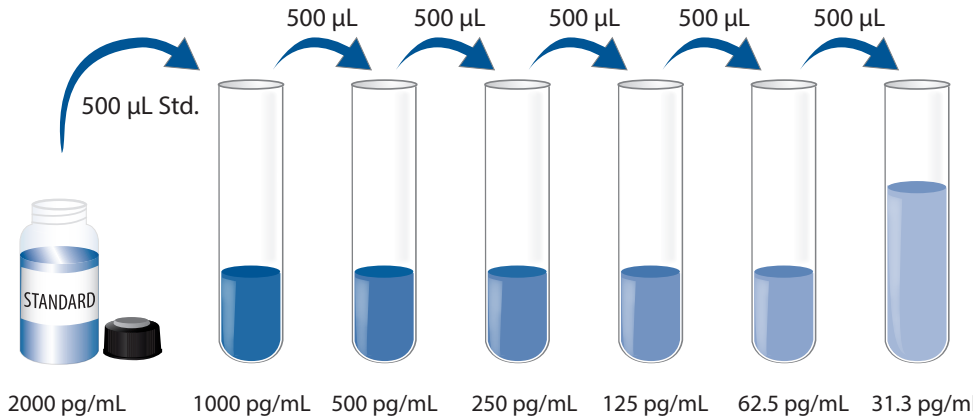
**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

**Human IL-11 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-11 Standard with Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6N (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**For Cell Culture Supernate Samples:** Pipette 500 µL of Calibrator Diluent RD5K into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).




**For Serum/Plasma Samples:** Pipette 500 µL of Calibrator Diluent RD6N into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-11 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD6N serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1S to each well.
4. Add 100 µL of standard, control, sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper toweling.
6. Add 200 µL of Human IL-11 Conjugate to each well. Cover with a new adhesive strip.
   **For Cell Culture Supernate Samples:** Incubate for 2.5 hours at room temperature.
   **For Serum/Plasma Samples:** Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

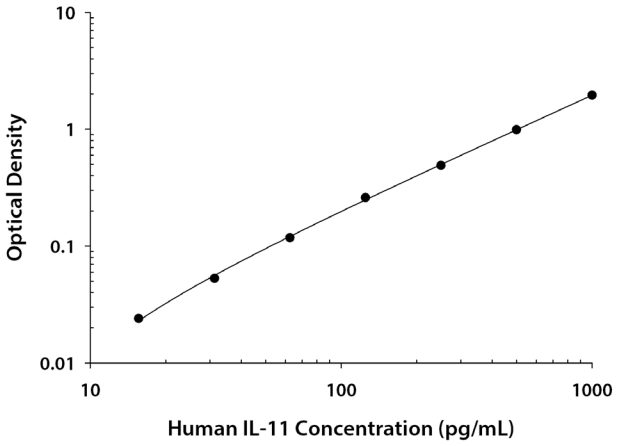
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-11 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

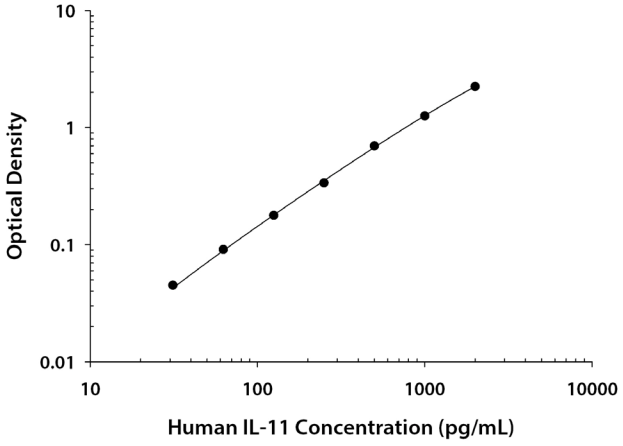
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.077<br>0.084 | 0.080 | — |
| 15.6 | 0.106<br>0.102 | 0.104 | 0.024 |
| 31.3 | 0.132<br>0.134 | 0.133 | 0.053 |
| 62.5 | 0.195<br>0.201 | 0.198 | 0.118 |
| 125 | 0.330<br>0.351 | 0.340 | 0.260 |
| 250 | 0.574<br>0.565 | 0.570 | 0.490 |
| 500 | 1.084<br>1.058 | 1.071 | 0.991 |
| 1000 | 2.047<br>2.016 | 2.032 | 1.952 |

### SERUM/PLASMA ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.061<br>0.061 | 0.061 | — |
| 31.3 | 0.107<br>0.105 | 0.106 | 0.045 |
| 62.5 | 0.152<br>0.153 | 0.152 | 0.091 |
| 125 | 0.235<br>0.243 | 0.239 | 0.178 |
| 250 | 0.400<br>0.396 | 0.398 | 0.337 |
| 500 | 0.773<br>0.742 | 0.758 | 0.697 |
| 1000 | 1.350<br>1.281 | 1.316 | 1.255 |
| 2000 | 2.358<br>2.244 | 2.301 | 2.240 |

## PRECISION

**Intra-assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

### CELL CULTURE SUPERNATE ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (pg/mL)** | 70.7 | 198 | 565 | 66.8 | 188 | 562 |
| **Standard deviation** | 1.7 | 5.2 | 11.8 | 3.4 | 16.8 | 37.2 |
| **CV (%)** | 2.4 | 2.6 | 2.1 | 5.1 | 8.9 | 6.6 |

### SERUM/PLASMA ASSAY

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (pg/mL)** | 50.0 | 320 | 1051 | 51.0 | 305 | 1018 |
| **Standard deviation** | 2.2 | 15.3 | 50.9 | 4.1 | 11.4 | 73.8 |
| **CV (%)** | 4.4 | 4.8 | 4.8 | 8.0 | 3.7 | 7.2 |

## RECOVERY

The recovery of human IL-11 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=5) | 98 | 90-108% |
| Serum (n=5) | 99 | 89-117% |
| EDTA plasma (n=5) | 103 | 97-110% |
| Heparin plasma (n=5) | 103 | 93-114% |
| Citrate plasma (n=5) | 105 | 96-118% |

## SENSITIVITY

The minimum detectable dose (MDD) of Human IL-11 is typically less than 8.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentrations of human IL-11 in various matrices and diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=5) | Serum (n=5) | EDTA plasma (n=5) | Heparin plasma (n=5) | Citrate plasma (n=5) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 99 | 100 | 93 | 98 | 94 |
| | Range (%) | 96-101 | 93-106 | 91-97 | 90-107 | 90-98 |
| 1:4 | Average % of Expected | 95 | 96 | 90 | 99 | 93 |
| | Range (%) | 91-98 | 89-102 | 87-95 | 90-110 | 88-98 |
| 1:8 | Average % of Expected | 94 | 98 | 90 | 99 | 92 |
| | Range (%) | 90-100 | 88-104 | 87-95 | 93-108 | 87-102 |
| 1:16 | Average % of Expected | 98 | 98 | 94 | 106 | 93 |
| | Range (%) | 87-108 | 87-104 | 86-110 | 95-116 | 86-100 |

## CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-11 produced at R&D Systems®.

The NIBSC/WHO IL-11 Reference Reagent 92/788 which is intended as a potency standard, was evaluated in this kit. The dose response of this reference material parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human IL-11 kit to approximate NIBSC 92/788 units, use the equation below.

NIBSC (92/788) approximate value (U/mL) = 0.011 x Quantikine® Human IL-11 value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma** - Thirty-four serum, EDTA plasma, heparin plasma, and citrate plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-11 in this assay. All samples measured less than the lowest Human IL-11 Standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

**Cell Culture Supernates** - MRC-5 human embryonic lung fibroblasts (1 x $10^6$ cells/mL) were cultured in DME supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate. Cells were unstimulated or stimulated with 10 ng/mL PMA and 1.0 ng/mL recombinant human IL-1α. Aliquots of the cell culture supernate were removed on day 2 and assayed for levels of human IL-11.

| Condition | Day 2 (pg/mL) |
|---|---|
| Unstimulated | 2400 |
| Stimulated | 10,000 |

## SPECIFICITY

This assay recognizes natural and recombinant human IL-11.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IL-11 control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant human:**

CNTF
gp130
IL-6
IL-6 R
LIF
OSM

**Recombinant mouse:**

IL-6
LIF

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