# Quantikine® ELISA

## Human IL-2 Immunoassay

Catalog Number D2050
S2050
PD2050

For the quantitative determination of human Interleukin 2 (IL-2) 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 2 (IL-2), also known as T cell growth factor (TCGF), is a 15-18 kDa variably glycosylated α-helical polypeptide that is a member of the Common gamma Chain (γc) cytokine family (1-4). It exists as a monomer and has a notably short half-life (< 30 minutes) (1). Human IL-2 is synthesized as a 153 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 133 aa mature region (5, 6). The mature region is α-helical in nature, and contains one utilized O-linked glycosylation site at Thr3 plus three cysteines, two of which form an intrachain disulfide bond that is essential for activity (7). Mature human IL-2 shares 73%, 66%, 78% and 97% aa identity with canine, rat, feline and rhesus monkey IL-2, respectively. Although human IL-2 shares only approximately 60% aa identity with the highly polymorphic mouse IL-2, human IL-2 is known to be active on mouse IL-2 responsive cells. Cells reported to secrete IL-2 include γδ T cells (8), activated conventional $CD4^+$ and $CD8^+$ T cells (1, 9), neurons (10, 11), microglia (12), and hematopoietic stem cells (13).

The receptor for IL-2 (IL-2 R) is composed of three subunits, the 55 kDa CD25/IL-2 Rα chain, the 70 kDa IL-2 Rβ chain, and the 65 kDa Common gamma Chain (1, 3). IL-2 first binds to CD25, the binary complex then recruits IL-2 Rβ and γc to form the quaternary signaling complex (1, 14). In addition to IL-2, IL-2 Rβ is used by IL-15 in its quaternary signaling complex. γc also serves as a signaling receptor for IL-4, -7, -9, -15, and -21 (1, 3).

*In vitro* studies have shown an important role for IL-2 in T cell activation and expansion. *In vivo*, IL-2 is critical for the development, maintenance and function of regulatory T cells (Treg) which provide protection against autoimmune disease. On the other hand, IL-2 can also promote autoimmune inflammation in target organs through its roles in regulating the expression of T cell trafficking genes, and production of Th2 cytokines. Within the $CD8^+$ T cell subset, IL-2 is essential for optimal primary responses and differentiation into terminal effector cells. IL-2 also promotes the development of activated $CD8^+$ T cells into memory cells. (1).

The Quantikine® Human IL-2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-2 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-2 and antibodies raised against recombinant human IL-2. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-2 showed dose response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-2.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-2 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-2 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 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 standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- 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 # | CATALOG # D2050 | CATALOG # S2050 | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human IL-2 Microplate** | 890042 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-2. | 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-2 Standard** | 890044 | 1 vial | 6 vials | Recombinant human IL-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.* | Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles. |
| **Human IL-2 Conjugate** | 890043 | 1 vial | 6 vials | 21 mL/vial of a polyclonal antibody specific for human IL-2 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1W** | 895117 | 1 vial | 6 vials | 11 mL/vial of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5-5** | 895485 | 1 vial | 6 vials | 21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.* | |
| **Calibrator Diluent RD6E** | 895017 | 1 vial | 6 vials | 21 mL/vial of animal serum with preservatives. *For serum/plasma samples.* | |
| **Wash Buffer Concentrate** | 895003 | 1 vial | 6 vials | 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL/vial of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

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

D2050 contains sufficient materials to run an ELISA on one 96 well plate.
S2050 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD2050). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

## 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.
- Human IL-2 Controls (optional; R&D Systems®, Catalog # QC01-1).

## PRECAUTIONS

Calibrator Diluent RD6E contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.

## REAGENT PREPARATION

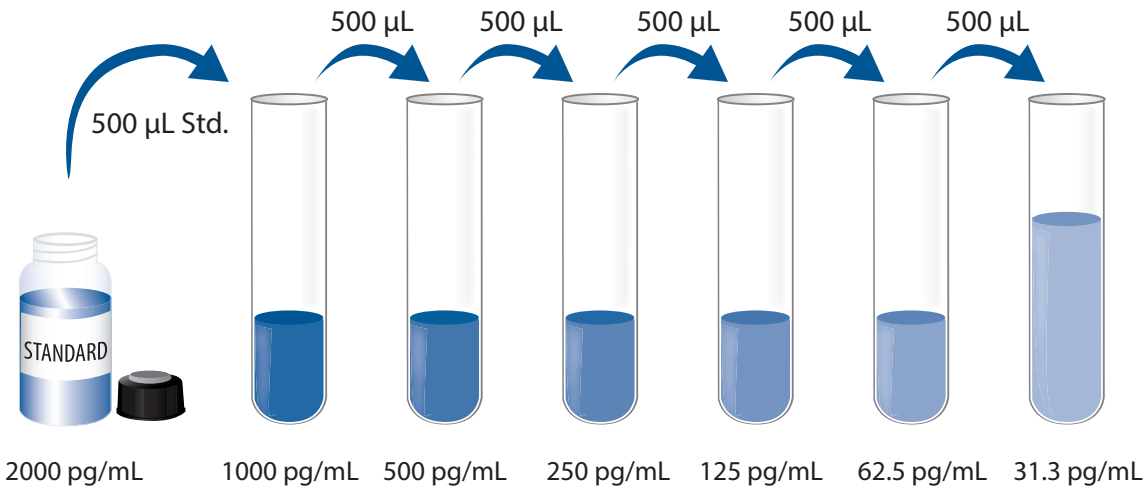
**Bring all reagents to room temperature before use.**

**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-2 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-2 Standard with of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6E (*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.

Pipette 500 µL of the Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6E (*for serum/plasma samples*) 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-2 Standard (2000 pg/mL) serves as the high standard. The appropriate calibrator diluent 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, samples, and working standards 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 RD1W to each well.
4. Add 100 µL of standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process twice for a total of three 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-2 Conjugate to each well. Cover with a new adhesive strip. 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 20 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.

## CALCULATION OF RESULTS

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

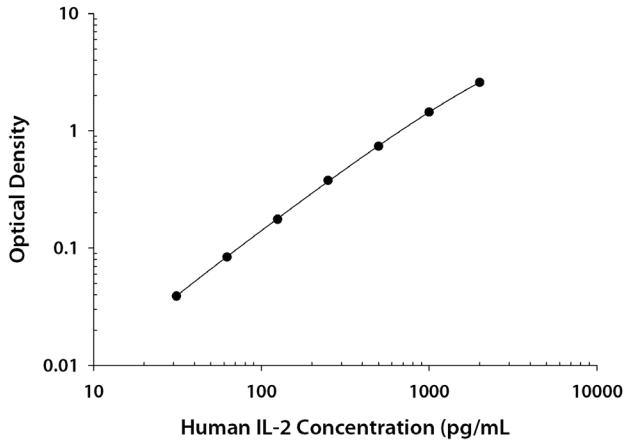
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-2 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

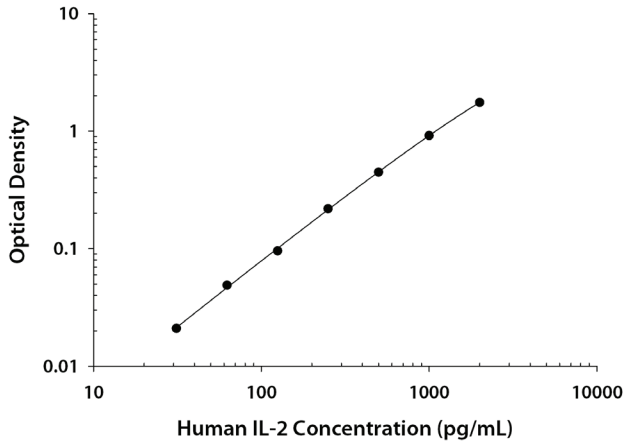
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.042<br>0.039 | 0.040 | —— |
| 31.3 | 0.081<br>0.077 | 0.079 | 0.039 |
| 62.5 | 0.127<br>0.120 | 0.124 | 0.084 |
| 125 | 0.221<br>0.210 | 0.216 | 0.176 |
| 250 | 0.404<br>0.431 | 0.418 | 0.378 |
| 500 | 0.802<br>0.756 | 0.779 | 0.739 |
| 1000 | 1.513<br>1.457 | 1.485 | 1.445 |
| 2000 | 2.734<br>2.527 | 2.630 | 2.590 |

### SERUM/PLASMA ASSAY




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.035<br>0.031 | 0.033 | —— |
| 31.3 | 0.054<br>0.054 | 0.054 | 0.021 |
| 62.5 | 0.084<br>0.081 | 0.082 | 0.049 |
| 125 | 0.121<br>0.137 | 0.129 | 0.096 |
| 250 | 0.247<br>0.258 | 0.252 | 0.219 |
| 500 | 0.474<br>0.489 | 0.482 | 0.449 |
| 1000 | 0.956<br>0.945 | 0.950 | 0.917 |
| 2000 | 1.785<br>1.799 | 1.792 | 1.759 |

## 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)** | 67.5 | 438 | 851 | 232 | 620 | 1148 |
| **Standard deviation** | 2.9 | 14.4 | 26.9 | 23.2 | 34.4 | 45.5 |
| **CV (%)** | 4.3 | 3.3 | 3.2 | 10.0 | 5.5 | 4.0 |

### 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)** | 98.0 | 619 | 1183 | 250 | 755 | 1470 |
| **Standard deviation** | 4.2 | 18.0 | 24.0 | 12.6 | 27.8 | 74.1 |
| **CV (%)** | 4.3 | 2.9 | 2.0 | 5.0 | 3.7 | 5.0 |

## RECOVERY

The recovery of human IL-2 spiked to three levels in samples throughout the range of the assay was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=5) | 101 | 96-107% |
| Serum (n=5) | 99 | 89-114% |
| EDTA plasma (n=5) | 118 | 104-139% |
| Heparin plasma (n=5) | 109 | 94-129% |
| Citrate plasma (n=5) | 105 | 98-111% |

## SENSITIVITY

The minimum detectable dose (MDD) of human IL-2 is typically less than 7 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, samples were spiked with high concentrations of human IL-2 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 | 102 | 97 | 100 | 104 | 100 |
| | Range (%) | 90-113 | 95-102 | 89-106 | 100-109 | 96-105 |
| 1:4 | Average % of Expected | 101 | 94 | 102 | 104 | 99 |
| | Range (%) | 90-111 | 91-97 | 97-107 | 99-108 | 96-102 |
| 1:8 | Average % of Expected | 99 | 87 | 99 | 100 | 97 |
| | Range (%) | 91-108 | 78-92 | 92-106 | 94-107 | 91-100 |
| 1:16 | Average % of Expected | —— | —— | 98 | 95 | 91 |
| | Range (%) | —— | —— | 88-114 | 86-109 | 85-100 |

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-2 produced at R&D Systems®.

NIBSC/WHO 1st International natural human IL-2 Standard 86/504, which was intended as a potency standard, the non-WHO interim reference recombinant human IL-2 Standard 86/564, and the 2nd International Standard recombinant human IL-2 86/500 were evaluated in this kit. All materials parallel the Quantikine® Human IL-2 standard curve. To convert sample values obtained with the Quantikine® Human IL-2 kit to relative approximate NIBSC units, use the appropriate equation below:

NIBSC (86/500) approximate value (IU/mL) = 0.017 x Quantikine® Human IL-2 value (pg/mL)

NIBSC (86/504) approximate value (IU/mL) = 0.016 x Quantikine® Human IL-2 value (pg/mL)

NIBSC (86/564) approximate value (U/mL) = 0.012 x Quantikine® Human IL-2 value (pg/mL)

**Note:** *Based on data generated in September 2008.*

## SAMPLE VALUES

**Serum/Plasma** - Thirty-seven serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-2 in this assay. All samples measured less than the lowest Human IL-2 Standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

## SPECIFICITY

This assay recognizes natural and recombinant human IL-2.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human IL-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

G-CSF
GM-CSF
IL-1α
IL-1β
IL-2 Rα
IL-2 Rβ
IL-2 Rγ
IL-3
IL-4
IL-6
IL-7
IL-8
LIF
TGF-β1
TGF-β2
TNF-α
TNF-β

### Recombinant mouse:

GM-CSF
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7

## REFERENCES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