

Human IL-2 ELISA Kit

For the Quantitative Determination of Human Interleukin 2 (IL-2)
Concentrations in Serum, Plasma, Cell Culture Supernatant, and
Other Biological Fluids.

Catalogue Number: EL10025

96 tests

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



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INTENDED USE

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

INTRODUCTION

Interleukin 2 (IL-2) is a lymphokine synthesized and secreted primarily by T helper lymphocytes that have been activated by stimulation with certain mitogens or by interaction of the T cell receptor complex with antigen/MHC complexes on the surfaces of antigen-presenting cells. The response of T helper cells to activation is induction of the expression of IL-2 and receptors for IL-2 and, subsequently, clonal expansion of antigen-specific T cells. At this level IL-2 is an autocrine factor, driving the expansion of the antigen-specific cells. IL-2 also acts as a paracrine factor, influencing the activity of other cells, both within the immune system and outside of it. B cells and natural killer (NK) cells respond to IL-2 when properly activated. The so-called lymphocyte activated killer, or LAK cells, appears to be derived from NK cells under the influence of IL-2.

Human IL-2 is a glycoprotein with an apparent molecular weight of 15,000 - 18,000. Natural IL-2 is glycosylated and varying degrees of glycosylation apparently account for the observed range of molecular weights seen on SDS-PAGE. Human IL-2 is synthesized as a polypeptide of 153 amino acid residues. The first 20 amino acids represent a signal sequence that is cleaved to produce the mature factor. The mature protein contains three cysteine residues, two of which form a disulfide bond that is required for biological activity. Murine IL-2 is approximately 63% identical to human IL-2, but contains a unique stretch of repeated glutamine residues. There is marked species cross-reactivity as human IL-2 has been found to be active on murine cell lines. Cells known to produce IL-2 include thymocytes, gamma delta T cells, B cells, CD4+ and CD8+ T cells, and neurons plus astrocytes.

IL-2 is a factor produced and secreted primarily by activated T helper cells that acts as an autocrine factor driving the expansion of antigen-specific cells and as a paracrine factor influencing the activity of a number of other cells including B cells, NK cells and LAK cells. A simplified but useful view of these activities is of lymphocytes expanding under the influence of IL-2 and becoming the target of other cytokines that cause their functional differentiation.

With respect to the specific role of IL-2 on the differentiation of T cells, the separation of CD4+ T helper cells into the categories of TH1 and TH2 according to their function in cell mediated or humoral immunity is a concept that is proving useful. In this system each category of cells secretes a characteristic set of cytokines that functions as a network to push the system either towards cellular immunity (delayed type hyper-sensitivity and cellular cytotoxicity) associated with TH1, or towards humoral immunity (antibody-

mediated) associated with TH2. IL-2, along with IFN-gamma and TNF-beta, is a defining product of the TH1 subset. Although the TH1 and TH2 subsets are relatively clearly defined in the murine immune system, these categories are not so clear-cut in the human immune system where the designations TH1-like and TH2-like have been suggested.

Other cells under the possible influence of IL-2 are neutrophils, monocytes, and gamma delta T cells, all of which demonstrate either activation, augmented function, or increased survival when exposed to IL-2. Finally, it should be mentioned that IL-2 is finding its way, along with many other cytokines, into the neurosciences as a possible neuromodulator and growth regulator of glial cells.

Because of the central role of the IL-2/IL-2R system in mediation of the immune response, it is obvious that monitoring and manipulation of this system has important diagnostic and therapeutic implications. IL-2 has shown promise as an anti-cancer drug by virtue of its ability to stimulate the proliferation and activities of tumor-attacking LAK and TIL (tumor-infiltrating lymphocytes) cells. However, problems with IL-2 toxicity are still of concern and merit investigation.

This IL-2 ELISA is a ready-to-use 4.5-hour solid phase immunoassay readily capable of measuring IL-2 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 2500 pg/mL. This assay has shown no cross-reactivity with other cytokines tested, and is expected to be used effectively for further investigations into the relationship between IL-2 and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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 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 colour develops in proportion to the amount of IL-2 bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

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

LIMITATIONS OF APPLICATION

- The Human IL-2 ELISA kit is not for use in clinical diagnostic procedures, and for laboratory use only.
- 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-2 MICROTITER PLATE (Part EL25-1) _____ 96 wells Pre-coated with anti-human IL-2 monoclonal antibody. | |
| 2. IL-2 Antibody Biotin Conjugate (Part EL25-2) _____ 7 mL Buffered protein base with preservative. | |
| 3. Avidin-HRP Conjugate (Part EL25-3) _____ 14 mL Polyclonal antibody against Human IL-2 conjugated to horseradish peroxidase. | |
| 4. IL-2 STANDARD (Part EL25-4) _____ 2 vials Recombinant human IL-2 (5 ng/vial) in a buffered protein base with preservative, lyophilized. | |
| 5. CALIBRATOR DILUENT I (Part EL25-5) _____ 25 mL Animal serum with buffer and preservative. <i>For serum/plasma testing.</i> | |
| 6. CALIBRATOR DILUENT II (Part EL25-6) _____ 25 mL Cell culture medium with calf serum and preservative. <i>For cell culture supernatant testing.</i> | |
| 7. WASH BUFFER (20X) (Part 30005) _____ 60 mL 20-fold concentrated solution of buffered surfactant. | |
| 8. SUBSTRATE A (Part EL25-7) _____ 10 mL Buffered solution with H ₂ O ₂ | |

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|-----|--|--------------|
| 9. | SUBSTRATE B (Part 30007) Buffered solution with TMB. | <u>10 mL</u> |
| 10. | STOP SOLUTION (Part 30008) 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material! | <u>15 mL</u> |

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 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 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

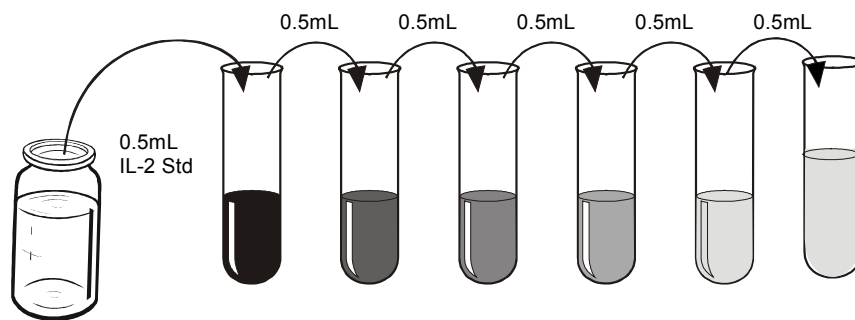
distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

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 Standard:**

- a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-2 Standard with 2.0 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 2500 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 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 (39 to 2500 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 stock solution will serve as the high standard (2500 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



| | | | | | | |
|---------------|------------|-----------|-------------|-------------|------------|----------|
| IL-2 Standard | 1250 pg/ml | 625 pg/ml | 312.5 pg/ml | 156.2 pg/ml | 78.1 pg/ml | 39 pg/ml |
| 2500 pg/ml | | | | | | |

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ASSAY PROCEDURE

1. Prepare Wash Buffer and IL-2 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 5 312.5 pg/mL (S5) |
| 1C, 1D | Standard 2 39 pg/mL (S2) | 2C, 2D | Standard 6 625 pg/mL (S6) |
| 1E, 1F | Standard 2 78.1 pg/mL (S3) | 2E, 2F | Standard 7 1250 pg/mL (S7) |
| 1G, 1H | Standard 3 156.2 pg/mL (S4) | 2G,2H | Standard 8 2500 pg/mL (S8) |

IL-2 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 Anti-IL-2 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 100 μ L Substrate Solution to 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 set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-2 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 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 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 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

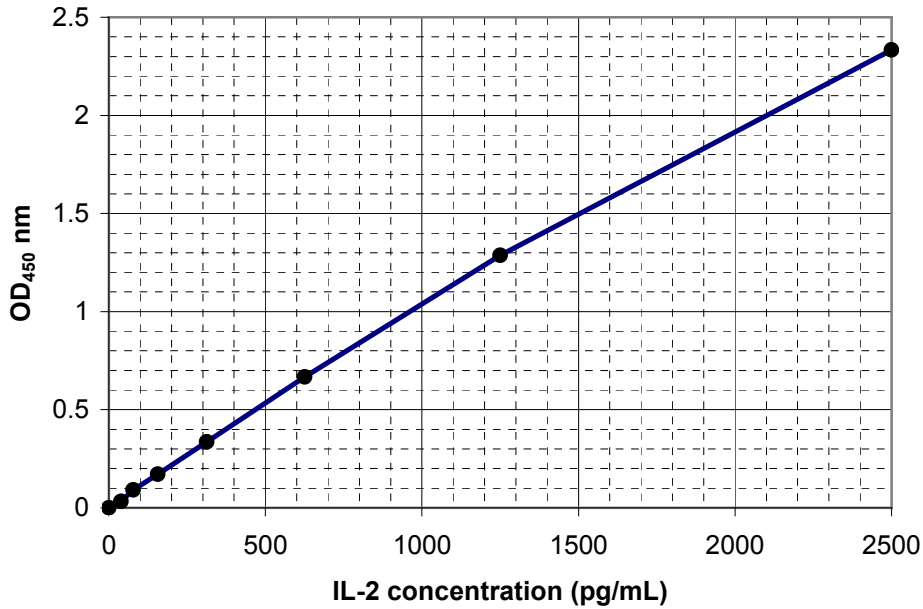
Results of a typical standard run of a IL-2 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not 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.

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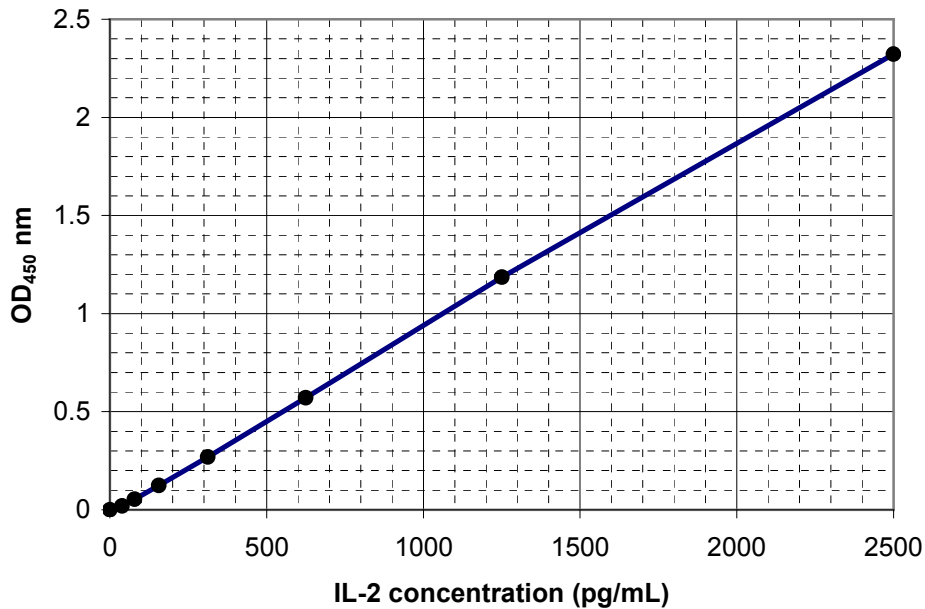
| Standard (pg/mL) | O.D. (450 nm) | Mean | Zero Standard Subtracted (Std.) - (S1) |
|------------------|---------------|-------|--|
| 0 | 0.061, 0.059 | 0.060 | 0 |
| 39.0 | 0.099, 0.088 | 0.093 | 0.033 |
| 78.1 | 0.155, 0.147 | 0.151 | 0.091 |
| 156.2 | 0.229, 0.233 | 0.231 | 0.171 |
| 312.5 | 0.384, 0.408 | 0.396 | 0.336 |
| 625 | 0.755, 0.699 | 0.727 | 0.667 |
| 1250 | 1.335, 1.359 | 1.347 | 1.287 |
| 2500 | 2.442, 2.344 | 2.393 | 2.333 |



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 | 0.072, 0.069 | 0.071 | 0 |
| 39.0 | 0.095, 0.087 | 0.091 | 0.020 |
| 78.1 | 0.128, 0.122 | 0.125 | 0.054 |
| 156.2 | 0.199, 0.191 | 0.195 | 0.124 |
| 312.5 | 0.350, 0.330 | 0.340 | 0.269 |
| 625 | 0.634, 0.648 | 0.641 | 0.570 |
| 1250 | 1.268, 1.246 | 1.257 | 1.186 |
| 2500 | 2.355, 2.431 | 2.393 | 2.322 |



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) | 81.5 | 305.8 | 1195.7 |
| Standard Deviation | 4.5 | 20.1 | 23.8 |
| <i>Coefficient of Variation (%)</i> | 5.5 | 6.6 | 2.0 |

| | <i>Calibrator Diluent II Assay</i> | | |
|-------------------------------------|------------------------------------|--------|--------|
| Sample | 1 | 2 | 3 |
| n | 20 | 20 | 20 |
| Mean (pg/mL) | 76.3 | 281.25 | 1098.2 |
| Standard Deviation | 5.3 | 12.6 | 63.5 |
| <i>Coefficient of Variation (%)</i> | 6.9 | 4.5 | 5.8 |

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 |

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| | <i>Calibrator Diluent I Assay</i> | | |
|-------------------------------------|-----------------------------------|-------|------|
| Sample | 1 | 2 | 3 |
| n | 20 | 20 | 20 |
| Mean (pg/mL) | 91.0 | 351.9 | 1280 |
| Standard Deviation | 4.6 | 20.3 | 25.9 |
| <i>Coefficient of Variation (%)</i> | 5.1 | 5.8 | 2.0 |

| | <i>Calibrator Diluent II Assay</i> | | |
|-------------------------------------|------------------------------------|-------|--------|
| Sample | 1 | 2 | 3 |
| n | 20 | 20 | 20 |
| Mean (pg/mL) | 93.8 | 345.6 | 1198.9 |
| Standard Deviation | 5.2 | 18.6 | 32.4 |
| <i>Coefficient of Variation (%)</i> | 5.5 | 5.4 | 2.7 |

3. RECOVERY

By employing various samples, the recovery of IL-2 was evaluated in 7 different amounts of IL-2 throughout the range of the assay. All samples were mixed and assayed in duplicate.

| <i>Sample Type</i> | <i>Average Recovery (%)</i> | <i>Range (%)</i> |
|--------------------|-----------------------------|------------------|
| Cell Culture Media | 91 | 87.0-95.0 |
| Serum | 99 | 92.0-110.0 |
| EDTA plasma | 115 | 110.0-125.0 |
| Heparin plasma | 110 | 105.0-121.0 |
| Citrate plasma | 106 | 97.0-108.0 |

4. SENSITIVITY

The minimum detectable quantities of human IL-2 as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 5.7 pg/mL and 7.1 pg/mL respectively. The two standard deviations above the mean optical density of the 20 replicates of the zero standard were defined as the minimum detectable quantities.

5. SPECIFICITY

This sandwich ELISA can detect both natural and recombinant human IL-2. This kit exhibits no significant cross-reactivity with human IL-1, IL-3, IL-4, IL-6, IL-7, IL-8, G-CSF, GM-CSF, TGF- β 1, TGF- β 2, TNF- α , TNF- β .

6. CALIBRATION

This immunoassay is calibrated against natural human IL-2. (NIBSC/WHO First International Standard 87/504).

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