human IL-2

Enzyme Immunometric Assay Kit

Catalog No. 900-118

96 Well Kit

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Description

Assay Designs' human IL-2 Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determintation of human IL-2 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to human IL-2 immobilized on a microtiter plate to bind the human IL-2 in the standards or sample. A recombinant human IL-2 Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a polyclonal antibody to human IL-2 is added. This antibody binds to the human IL-2 captured on the plate. After a short incubation the excess antibody is washed out and goat anti-rabbit IgG conjugated to Horseradish peroxidase is added, which binds to the polyclonal human IL-2 antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of human IL-2 in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Interleukin-2 (IL-2), also known as T-cell growth factor, plays a critical role in the mediation of immune response by acting as a growth and differentiation factor for T cells[3], as well as B cells, natural killer (NK) cells, lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TIL), monocytes, macrophages, and oligodendrocytes. Before it becomes biologically active, the protein undergoes extensive post-translational modification including cleavage, glycosylation and the formation of disulfide bonds. Portions of IL-2 are highly conserved across species in areas involved in receptor binding during activated complex formation. The human IL-2 receptor can be composed of several combinations of a 55 kDa IL-2Rα subunit, a 70-75 kDa IL-2Rβ subunit, and a 64 kDa IL-2Rγ subunit. IL-2 binding affinity varies depending on which combination of subunits is present in the bonding complex, with the high affinity binding site having all three subunits[4]. Studies have shown IL-2 to cause the regression of some types of tumors and it may have implications in immunotherapy and the treatment of infectious diseases and immune deficiencies[5].

Precautions

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1. Stop Solution 2 is a 1 normal (1N) hydrochloric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The human IL-2 Standard provided, Catalog No. 80-0641, should be handled with care because of the known and unknown effects of human IL-2.
5. The human IL-2 Standard should be stored at or below -20 °C.

Materials Supplied

1. **human IL-2 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1168**
   A plate using break-apart strips coated with monoclonal antibody specific to human IL-2.
2. **human IL-2 Antibody, 10 mL, Catalog No. 80-0311**
   A yellow solution of rabbit polyclonal antibody to human IL-2.
3. **Assay Buffer 13, 55 mL, Catalog No. 80-1500**
   Tris buffered saline containing proteins and detergents.
4. **human IL-2 Conjugate, 10 mL, Catalog No. 80-1167**
   A blue solution of goat anti-rabbit antibody conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**
   Tris buffered saline containing detergents.
6. **human IL-2 Standard, 2 each, Catalog No. 80-0641**
   Two vials containing 2,000 pg each of lyophilized recombinant human IL-2.
7. **TMB Substrate, 10 mL, Catalog No. 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use. **Protect from prolonged exposure to light.**
8. **Stop Solution 2, 10 mL, Catalog No. 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **human IL-2 Assay Layout Sheet, 1 each, Catalog No. 30-0200**
10. **Plate Sealer, 3 each, Catalog No. 30-0012**

Storage

All components of this kit, **except the Standards**, are stable at 4°C until the kit's expiration date. The Standards **must** be stored at or below -20°C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 100 µL and 1,000 µL.
3. Disposable test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 100 µL.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. A microplate shaker
8. Adsorbent paper for blotting.
9. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
10. Graph paper for plotting the standard curve.

Sample Handling

Assay Designs' human IL-2 EIA is compatible with human IL-2 samples in a wide range of matrices. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions.

Culture fluids and serum are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of human IL-2 in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive human IL-2. If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at -70 °C to avoid loss of bioactive human IL-2. Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
9. **It is important that the matrix for the standards and samples be as similar as possible. Human IL-2 samples diluted with Assay Buffer 13 should be run with a standard curve diluted in the same buffer. Tissue culture samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step #2.**

Reagent Preparation

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **human IL-2 Standards**
   Allow the 2,000 pg human IL-2 standard to warm to room temperature. Reconstitute one vial of 2,000 pg human IL-2 Standard with 2 mL standard diluent (Assay Buffer 13 or Tissue Culture Media) for a 1,000 pg/mL stock vial. Vortex thoroughly, wait 5 minutes and vortex again prior to use.

   Label seven disposable 12 x 75 mm tubes #1 through #7. Pipet 500 µL standard diluent into each tube. Remove 500 µL from reconstituted stock vial and add to tube #1 and vortex thoroughly. Remove 500 µL from tube #1 and add to tube #2. Vortex thoroughly. Continue this for tubes #3 through #7.

   The concentration of human IL-2 in tubes #1 through #7 will be 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 pg/mL respectively. See human IL-2 Assay Layout Sheet for dilution details. STORE STANDARD AT -20 °C.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100 µL of standard diluent (Assay Buffer 13 or Tissue Culture Media) into the S0 (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100 µL of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
10. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 100 µL of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
14. Pipet 100 µL of Substrate Solution into each well.
15. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
16. Pipet 100 µL Stop Solution to each well.
17. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of human IL-2 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of human IL-2 can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the Average Net OD for each standard versus human IL-2 concentration in each standard. Approximate a straight line through the points. The concentration of human IL-2 in the unknowns can be determined by interpolation.

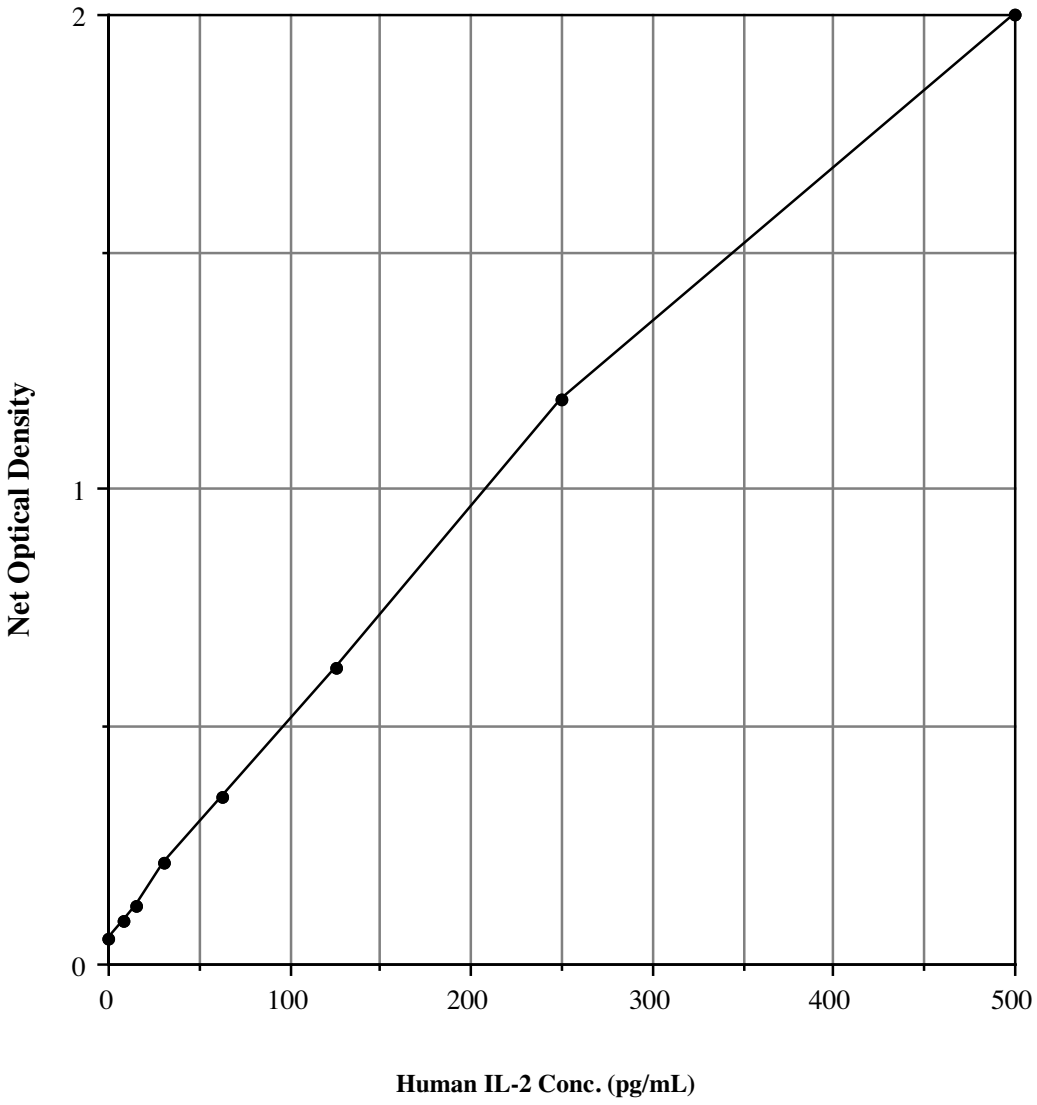
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	**human IL-2 (pg/mL)**
Blank	(0.073)		
S0	0.124	0.051	**0**
S1	2.073	2.000	**500**
S2	1.262	1.189	**250**
S3	0.697	0.624	**125**
S4	0.424	0.351	**62.5**
S5	0.285	0.212	**31.25**
S6	0.196	0.123	**15.63**
S7	0.165	0.092	**7.81**
Unknown 1	2.013	1.940	**478**
Unknown 2	0.625	0.552	**105**

Typical Standard Curve

A typical standard curve in Assay Buffer 13 is shown below. This curve **must not** be used to calculate human IL-2 concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[6].

Sensitivity

Sensitivity was calculated in Assay Buffer 13 by determining the average optical density bound for twenty (20) wells run with 0 pg/mL Standard, and comparing to the average optical density for twenty (20) wells run with Standard #7. The detection limit was determined as the concentration of human IL-2 measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 =	0.064 ± 0.005 (7.7%)
Mean OD for Standard #7 =	0.104 ± 0.009 (8.2%)
Delta Optical Density (7.81 - 0 pg/mL) = 0.104 - 0.064 =	0.040
2 SD's of 0 pg/mL Standard = 2 x 0.005 =	0.010
Sensitivity = $\frac{0.010}{0.040}$ x 7.81 pg/mL =	**2.0 pg/mL**

Linearity

A sample containing 393 pg/mL human IL-2 was serially diluted 4 times 1:2 in the Assay Buffer 13 supplied in the kit and measured in the assay. The data was plotted graphically as actual human IL-2 concentration versus measured human IL-2 concentration.

The line obtained had a slope of 1.032 with a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containging low, medium and high concentrations of human IL-2 and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of human IL-2 in multiple assays (n=12).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of human IL-2 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	human IL-2 (pg/mL)	Intra-assay % CV	Inter-assay % CV
Low	104	3.4	
Medium	203	2.6	
High	438	2.6	
Low	101		5.0
Medium	196		4.8
High	454		6.3

Cross Reactivities

The cross reactivities for a number of related compounds were determined by dissolving the cross reactants in Assay Buffer at a concentration of 5,000 pg/mL. These samples were then measured in the human IL-2 assay.

Compound	Cross Reactivity
human IFN-γ	0.98%
human IL-6	0.46%
mouse IL-2	<0.01%
human IL-1β	<0.01%
human IL-4	<0.01%
human TNF-α	<0.01%
human GM-CSF	<0.01%

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Human IL-2 concentrations were measured in human serum, human heparized plasma and Tissue Culture Media. Human IL-2 was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Human Serum	103.4	1:8
Human Heparized Plasma	98.1	1:4
TCM + 10% FBS	108.0	1:2

* See Sample Handling instructions on page 4 for details.

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques, 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. T.R. Mosmann, et al., Immunol. Today, (1987) 8 (7-8): 223-227.
4. S.L. Gaffen, et al., "The Cytokine Handbook 4th Ed.", (1988) San Diego: Academic Press.
5. B.E. Landgraf, et al., J. Biol. Chem., (1992) 267 (26): 18511-18519.
6. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

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Material Safety Data Sheet (MSDS) available on our website or by fax.

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Catalog No. 25-0520

January 26, 2007