IL-2 (human), ELISA kit

Catalog # ADI-900-118A
For use with serum, plasma and culture supernates

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

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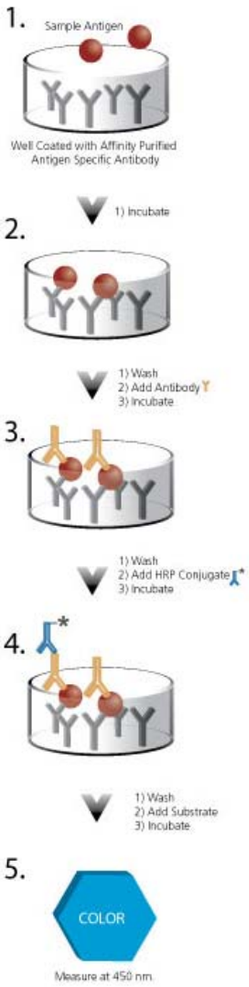


Introduction

The IL-2 (human), ELISA kit is a complete kit for the quantitative determintation of human IL-2 in culture supernates, plasma, and serum. Please read the complete kit insert before performing this assay.

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[1], 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[2]. 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[3].

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody specific for human IL-2. The plate is then incubated.
2. The plate is washed, leaving only bound IL-2 on the plate. A yellow solution of polyclonal antibody to human IL-2 is then added. This binds the IL-2 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the human IL-2 polyclonal antibody. The plate is again incubated.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of human IL-2 in the sample.

Materials Supplied

Do not mix components from different kit lots or use reagents beyond the kit's expiration date.

Activity of conju-gate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

The standard should be handled with care due to the

Stop solution is caustic. Keep tightly capped.

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**

Reagents require separate storage conditions.

Storage

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

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 570nm and 590 nm.
10. Graph paper for plotting the standard curve.

Reagent Preparation

Bring all reagents to room tempera-ture for at least 30 minutes prior to opening.

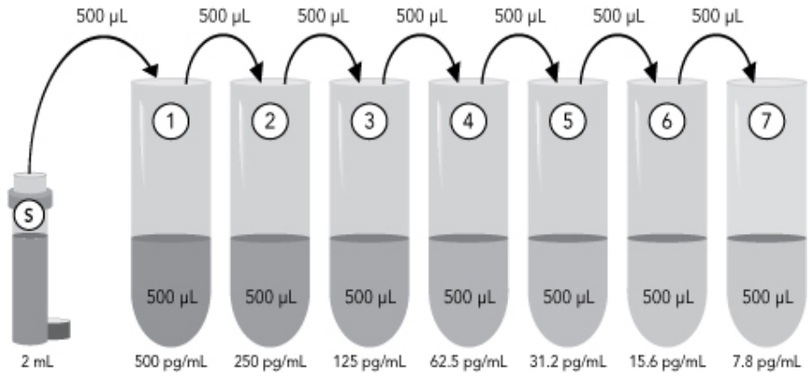
Standards can be made up in either glass or plastic tubes.

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 thorouaghly. 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 image below). Standards should be used within 60 minutes of preparation.



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Sample Handling

The IL-2 (human), ELISA kit is compatible with human IL-2 samples in culture supernates, plasma and serum. Samples diluted sufficiently into the assay buffer can be read directly from a standard curve. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. A minimum 1:2 dilution is recommended serum, plasma, and culture supernates (with or without 10% serum supplement). These are the minimum recommended dilutions to remove matrix interference in the assay.

Samples in the majority of culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the culture media instead of Assay Buffer. Culture media must be diluted at least 1:2 in the assay buffer to remove matrix interference. 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.

Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity is observed. Non-conditioned Dulbecco's Modified Eagle's Medium (DMEM) with or without 10% fetal bovine serum (FBS) was spiked with recombinant human IL-2 and diluted in the assay buffer. The assay buffer was spiked to the same concentration and used as a control to determine linearity of the culture medium. Pools of Human heparin plasma were diluted in the assay buffer to produce values within the dynamic range of the assay.

Dilution	Culture Media (DMEM)	Culture Media (DMEM +10% FBS)	Serum	Human Heparin Plasma
1:2	102	106	107	84
1:4	105	100	110	90
1:8	108	104	109	94
1:16	121	113	107	90
1:32	172	154	100	100

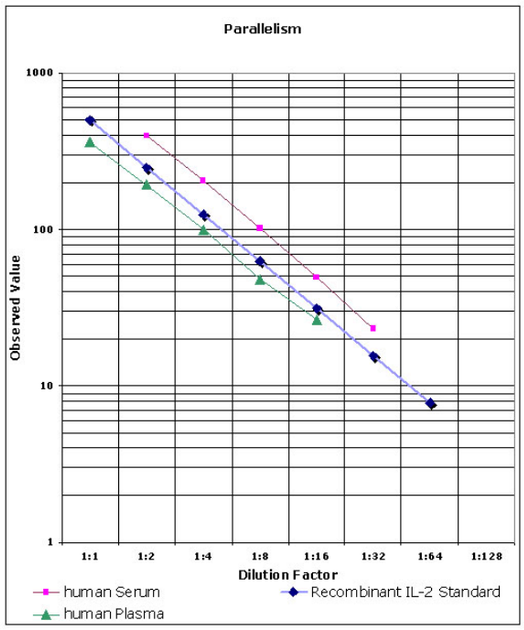
Spike and Recovery

After diluting each sample matrix to its minimum required dilution, recombinant human IL-2 was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range of percent recovery at the three concentrations are indicated below for each matrix.

Sample Matrix	Minimum Required	Spike Concentration	Recovery of Spike
Serum (n = 5)	1:2	346	87% (78-94%)
		248	76% (68-81%)
		53	59% (36-81%)
Human Heparin Plasma (n=2)	1:2	346	83% (74-92%)
		248	72% (69-75%)
		53	55% (53-56%)
TCM + 10% FBS (n=1)	1:2	400	103% (n/a)
		200	105% (n/a)
		50	112% (n/a)

Parallelism

A parallelism experiment was carried out to determine if the recombinant human IL-2 standard accurately determines human IL-2 concentrations in biological matrices. To assess parallelism, values for the sample matrices shown in the graph were obtained from a standard curve using four parameter logistic curve fitting. The observed concentration was plotted against the dilution factor. Parallelism of the curves demonstrates that the antibody binding characteristics are similar enough to allow the accurate determination of analyte levels in diluted samples.



Serum and Plasma Preparation

1. Collect whole blood in appropriate tubes for either serum or plasma.
2. Allow serum to clot for 30 minutes.
3. Centrifuge at 1000 x g for 15 minutes at 4˚C.
4. Place supernatants in a clean tube.
5. The supernatant may be aliquoted and stored at or below -20˚C, or used immediately in the assay.
6. Avoid repeated freeze-thaw cycles.

Assay Procedure

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the standards and samples to the bottom of the wells.

Pipet the reagents to the sides of the wells to avoid possible contamination.

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.

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.

1. Pipet 100 µL of standard diluent (Assay Buffer 13 or Culture Media) into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
5. 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.
6. Pipet 100 µL of yellow Antibody into each well, except the Blank.
7. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µL of blue Conjugate to each well, except the Blank.
10. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5).Pipet 100 µL of Substrate Solution into each well.
12. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Pipet 100 µL Stop Solution to each well.
14. Zero 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

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

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

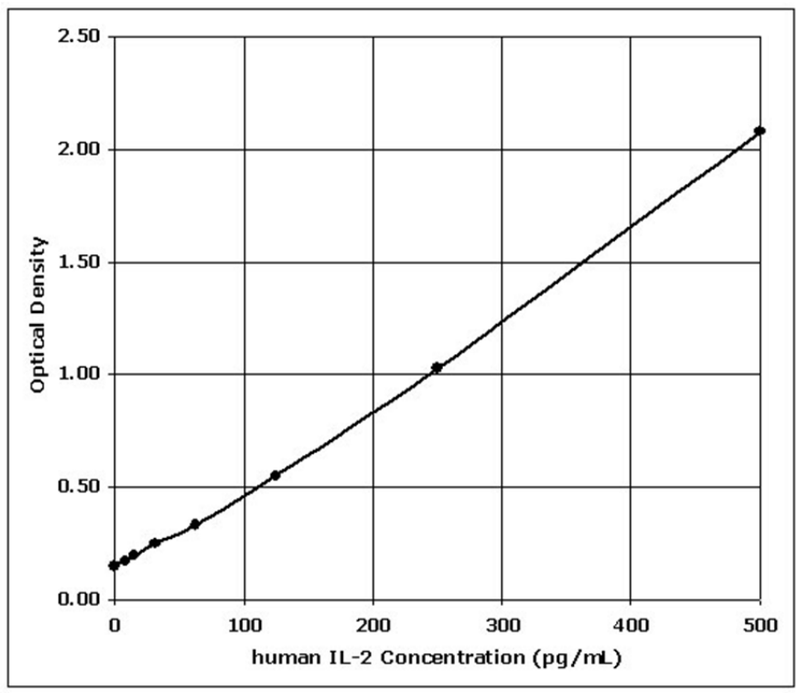
2. Using linear graph paper, plot the Average Net OD for each standard versus human IL-2 concentration in each standard. Approximate a straight line through the points. The concentration of human IL-2 in the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

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	(pg/mL)
Blank	(0.040)		
S0	0.188	0.148	0
S1	2.117	2.077	500
S2	1.068	1.028	250
S3	0.586	0.546	125
S4	0.371	0.331	62.5
S5	0.285	0.245	31.25
S6	0.234	0.194	15.63
S7	0.212	0.172	7.81
Unknown 1	2.030	1.990	480
Unknown 2	0.534	0.494	109



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

The sensitivity of the assay, defined as the concentration of human IL-2 measured at 2 standard deviations from the mean of 20 zeros along the standard curve, was determined to be 6.6 pg/mL.

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing human IL-2 in a single assay.

pg/mL	%CV
431	1.8
175	3.9
84	2.8

Inter-assay precision was determined by measuring buffer controls of varying human IL-2 concentrations in multiple assays over several days.

pg/mL	%CV
501	12.5
209	10.5
108	9.4

Specificity

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 IL-6	<0.01%
mouse IL-2	<0.01%
human TNF-α	<0.01%

References

1. T.R. Mosmann, *et al*., Immunol. Today, (1987) 8 (7-8): 223-227.
2. S.L. Gaffen, *et al*., “The Cytokine Handbook 4th Ed.”, (1988) San Diego: Academic Press.
3. B.E. Landgraf, *et al*., J. Biol. Chem., (1992) 267 (26): 18511-18519.

Notes

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Catalog Number: 25-0520 Revision: 01/14/2015