



IL-6 (human), ELISA kit

Catalog # ADI-901-033

5x96 well enzyme-linked immunosorbent assay kit
For use with serum, plasma , culture supernates, and urine



Reagents require separate storage conditions.

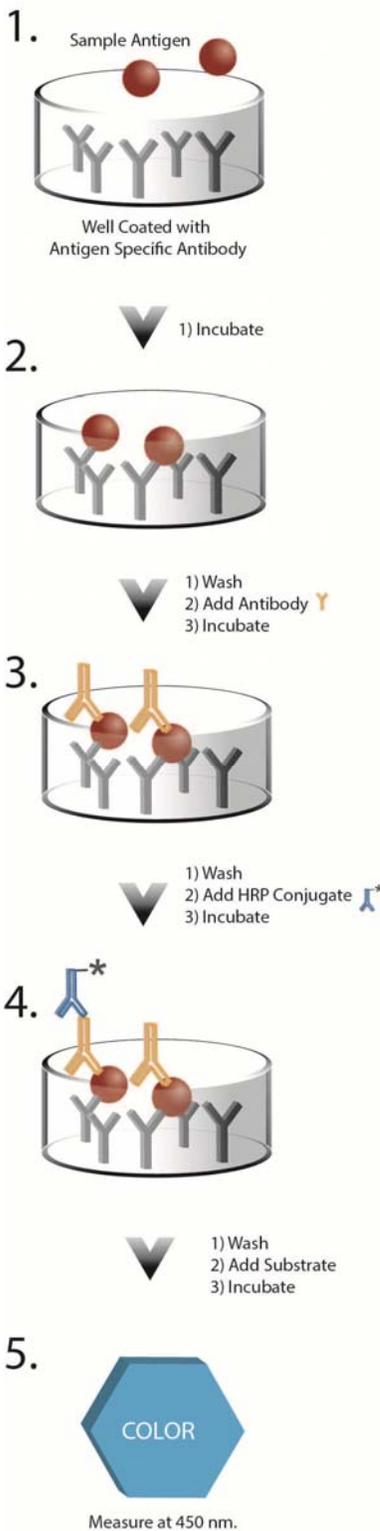


Check our website for additional protocols, technical notes and FAQs.

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Introduction

The IL-6 (human), ELISA kit is a complete kit for the quantitative determination of human IL-6 in serum, plasma, culture supernates, and urine. Please read the complete kit insert before performing this assay.

Interleukin-6 (IL-6) is a cytokine critical to the regulation of the immune and hematopoietic systems. It has been called interferon β 2, hybridoma/plasmacytoma growth factor, B-cell stimulatory factor 2, 26kDa inducible protein, hepatocyte stimulating factor, hematopoietic colony stimulating factor, monocyte granulocyte inducer type 2 and cytotoxic T-cell differentiation factor^{1, 2, 7}. A 212-amino acid glycoprotein, human IL-6 is expressed by cell types such as T-cells, mast cells, monocytes, macrophages, fibroblasts, endothelial cells, keratinocytes, and many tumor cell lines. It appears to take part in acute phase reactions and response to injury and inflammation^{1,2,6}. Human IL-6 also stimulates the differentiation of B-cells for antibody production, promotes expansion of activated T-cells, expands hematopoietic cell production, and induces the expression of acute phase proteins²⁻⁷. Levels of human IL-6 have been shown to increase rapidly in serum with sepsis and burn trauma, and in cerebrospinal fluid with acute viral or bacterial infection of the CNS⁶. Human IL-6 is implicated in conditions including autoimmune diseases and polyclonal B-cell abnormalities^{1,6}.

Principle

1. Samples and standards are added to wells coated with a monoclonal antibody specific for human IL-6. The plate is then incubated.
2. The plate is washed, leaving only bound IL-6 on the plate. A yellow solution of polyclonal antibody to human IL-6 is then added. This binds the IL-6 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-6 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-6 in the sample.



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



Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.



The standard should be handled with care due to the known and unknown effects of the antigen.



Protect substrate from prolonged exposure to light.



Stop solution is caustic. Keep tightly capped.

Materials Supplied

1. **human IL-6 Microtiter Plate, Five Plates of 96 Wells**
Catalog No., 80-0318
Clear plates using break-apart strips coated with monoclonal antibody specific to human IL-6
2. **IL-6 (human) Antibody , 50 mL**
Catalog No. 80-2045
A yellow solution of rabbit polyclonal antibody to human IL-6
3. **Assay Buffer 13 Concentrate, 30 mL**
Catalog No., 80-2023
A 10x solution of tris buffered saline containing proteins and detergents
4. **IL-6 (human) Conjugate, 50 mL**
Catalog No., 80-2046
A blue solution of donkey anti-rabbit IgG conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 250 mL**
Catalog No., 80-1799
Tris buffered saline containing detergents.
6. **human IL-6 Standard, 7 each**
Catalog No., 80-0625
Seven vials, each containing 5,000 pg of lyophilized recombinant human IL-6. **Avoid repeated freeze/thaw cycles.**
7. **TMB Substrate, 50 mL**
Catalog No., 80-1805
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.
8. **Stop Solution 2, 50 mL**
Catalog No., 80-1804
A 1N solution of hydrochloric acid in water. Keep tightly capped.
9. **human IL-6 Assay Layout Sheet, 1 each**
Catalog No., 80-0096.
10. **Plate Sealer, 5 each**
Catalog No., 30-0012



Reagents require separate storage conditions.

Storage

All components of this kit, **except the human IL-6 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 μ L and 1,000 μ L.
3. Disposable test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 100 μ L.
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.



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 bio-active analyte. Repeated freeze/thaw cycles should be avoided.

Sample Handling

The IL-6 (human), ELISA kit is compatible with human IL-6 samples in culture fluids, serum, plasma, and urine. Samples diluted sufficiently into the assay buffer can be read directly from the 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 1:4 dilution is recommended for serum and urine samples, and a minimum 1:8 dilution for plasma. These are the recommended dilutions to remove matrix interference in the assay. A different dilution may be needed to bring samples within the dynamic range of the assay or to remove matrix interference. The optimal dilution should be determined by the investigator.

Samples in the majority of culture media, including those containing fetal bovine serum, can be read in the assay, provided the standards have been diluted into the diluted non-conditioned culture media instead of Assay Buffer 13. Culture media must be diluted at least 1:2 in Assay Buffer 13 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-6 in the appropriate matrix.

Sample Recoveries

Human IL-6 concentrations were measured in a variety of different samples including tissue culture media, human serum, EDTA plasma and urine. Human IL-6 was spiked into samples of these matrices that were diluted with the assay buffer and assayed in the kit. The following results were obtained:

Sample	%Recovery *	Recommended Dilution*
Tissue Culture Media	104.9	1:2
Human Serum	88.3	1:4
Human EDTA Plasma	100.1	1:8
Human Urine	91.9	1:4



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



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

Reagent Preparation

1. **Wash Buffer**

Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first.

2. **Assay Buffer**

Just before use, prepare the amount of assay buffer needed by making a 1:10 dilution. As an example, dilute 10 mL of the supplied Assay Buffer 13 Concentrate with 90 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

3. **human IL-6 Standards**

Reconstitute one vial of human IL-6 Standard with 500 μ L of standard diluent (Assay Buffer 13 or Culture Media). Mix thoroughly without foaming.

Label seven 12x75mm glass tubes #1 through #7. Pipet 950 μ L of standard diluent into tube #1. Pipet 500 μ L of standard diluent into tubes #2 through #7. Add 50 μ L of the 10,000 pg/mL Standard to tube #1. Vortex thoroughly. Add 500 μ L of tube #1 to tube #2 and vortex thoroughly. Add 500 μ L of tube #2 to #3 and vortex thoroughly. Continue this for tubes #4 through #7.

The concentration of human IL-6 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-6 Assay Layout for dilution details. Diluted standards should be used within 1 hour of preparation.



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



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



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



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



Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Assay Procedure

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 pouch and seal the ziploc. Store unused wells at 4°C.

1. Pipet 100 μ L of standard diluent (Assay Buffer 13 or Culture Media) into the S0 (0 pg/mL standard) wells.
2. Pipet 100 μ L of Standards #1 through #7 to the bottom of the appropriate wells.
3. Pipet 100 μ L of the Samples to the bottom of the appropriate wells.
4. Seal the plate. Incubate at room temperature, on a plate shaker for 1 hour at ~500rpm.
5. Empty the contents of the wells and wash by adding 400 μ L 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 μ L 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 ~500rpm.
8. Wash as above (Step 5).
9. Add 100 μ L 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 ~500rpm.
11. Wash as above (Step 5).
12. Pipet 100 μ L of Substrate Solution into each well.
13. Incubate for 15 minutes on a plate shaker, at room temperature at ~500rpm.
14. Pipet 100 μ L Stop Solution 2 to each well. This stops the reaction and the plate should be read immediately.
15. After zeroing the plate reader against the substrate blank, read the optical density at 450 nm preferably with correction between 570 and 590 nm. If the plate reader is not able to be zeroed against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.



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

Calculation of Results

Several options are available for the calculation of the concentration of human IL-6 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-6 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.

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

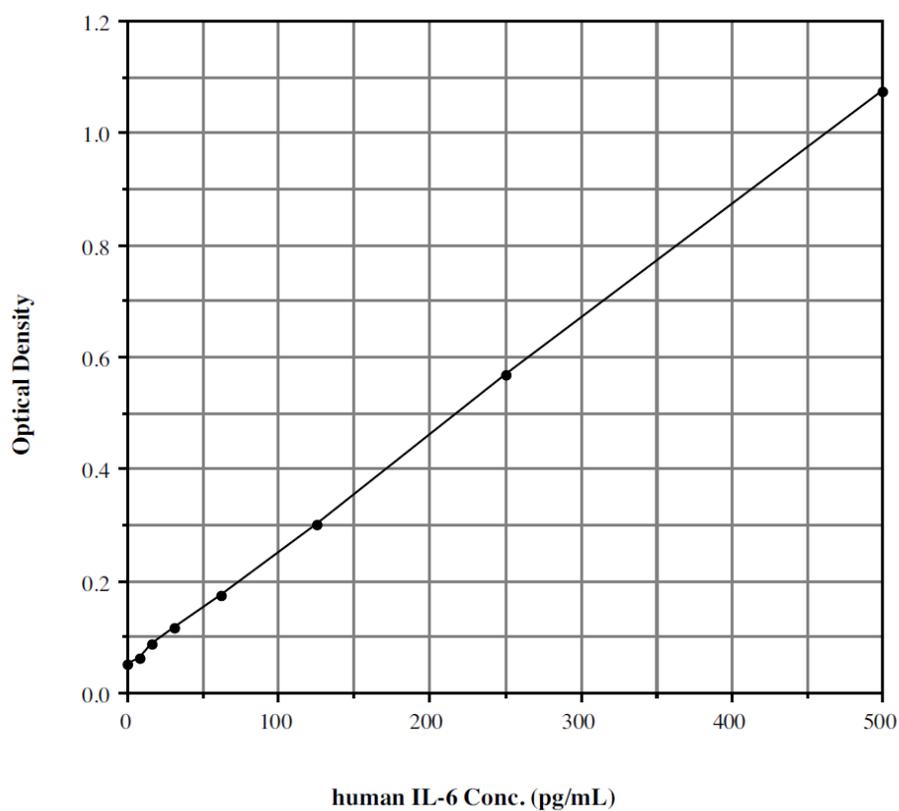
2. Using linear graph paper, plot the Average Net OD for each standard versus human IL-6 concentration in each standard. Approximate a straight line through the points. The concentration of human IL-6 in the unknowns can be determined by interpolation.

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	h IL-6 (pg/mL)
Blank	0.055		
S0	0.107	0.052	0
S1	1.128	1.073	500
S2	0.622	0.567	250
S3	0.356	0.301	125
S4	0.230	0.175	62.5
S5	0.172	0.117	31.25
S6	0.140	0.085	15.63
S7	0.118	0.063	7.81
Unknown	0.356	0.301	125.24
Unknown	0.262	0.207	78.62



Performance Characteristics

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with 0 pg/mL Standard, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of human IL-6 measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 = 0.047 ± 0.005 (9.7%)

Mean OD for Standard #7 = 0.060 ± 0.004 (6.0%)

Delta Optical Density = (7.81 - 0 pg/mL) = 0.060 - 0.047 = 0.013

2 SD's of 0 pg/mL Standard = 2 x 0.005 = 0.010

Sensitivity = $\frac{0.010}{0.013} \times 7.81 \text{ pg/mL} = 6.01 \text{ pg/mL}$

Linearity

A sample containing 274.6 pg/mL mouse IL-6 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-6 concentration versus measured human IL-6 concentration.

The line obtained had a slope of 0.9211 with a correlation coefficient of 0.9917.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of human IL-6 and running these samples multiple times (n=16) in the same assay.

Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of human IL-6 in multiple assays (n=9).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of human IL-6 determined in these assays as calculated by a 4 parameter logistic curve fitting program was determined in by assaying 20 replicates of three buffer controls in a single assay.

	Average OD	Net OD	h IL-6 (pg/mL)
Low	109.8	3.8	
Medium	166.8	5.7	
High	250.7	6.4	
Low	95.2		14.4
Medium	186.6		12.6
High	256.8		10.3

Cross Reactivities

The human IL-6 ELISA Kit is specific for bioactive human IL-6. The kit is unaffected by the presence of recombinant mouse IL-6 or recombinant human IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-7, IL-8, TNF- α , TNF- β , GM-CSF and G-CSF.

References

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