Interleukin-6 (human) ELISA Kit

Item No. 501030

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TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401032	Anti-IL-6 (human) ELISA Strip Plate	1 plate
401030	Anti-IL-6 (human) Biotin Conjugate	1 vial/100 dtn
483364	IL-6 (human) ELISA Standard	1 vial/10 ng
401033	IL-6 (human) Streptavidin-HRP	2 vials/1.5 ml
400054	Immunoassay Buffer B Concentrate (10X)	2 vials/10 ml
400035	Polysorbate 20	1 vial/3 ml
400012	96-Well Cover Sheet	1 cover
400074	TMB Substrate Solution	1 vial/12 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
10011355	HRP Stop Solution	1 vial/12 ml

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Interleukin-6 (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab-coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
E-Mail:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or deionized water is acceptable
4. Materials used for **Sample Preparation** (see page 9)

INTRODUCTION

Background

Interleukin-6 (IL-6) is a 212 amino acid polypeptide produced by multiple cell types, including monocytes, macrophages, and T cells. The molecular weight varies from 21-29 kDa due to extensive and variable phosphorylation and glycosylation.[1] Post-translational modifications may be tissue specific and may also determine biological activity.[2]

IL-6 is involved in many defense mechanisms including the immune response, hematopoiesis, bone metabolism, and acute phase reactions.[3] IL-6 promotes the proliferation of activated B-cells, acts as a B-cell differentiation factor, and stimulates the secretion of immunoglobulins.[3,4] IL-6 also induces the differentiation of naïve T cells into TH17 effector cells.[5]

Elevated levels of IL-6 have been detected in a number of disease states, including bacterial and viral infections, HIV infections, autoimmune diseases, and some cancers.[6]

About This Assay

Cayman's IL-6 (human) ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA that permits IL-6 measurements within the range of 3.9-250 pg/ml, with an LLOQ of 7.8 pg/ml.

Description of Immunometric ELISAs

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a rat monoclonal antibody specific for human IL-6. This antibody will bind any IL-6 introduced into the well. Standards or biological test samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of a second, non-overlapping biotin-conjugated rat monoclonal antibody specific for human IL-6 that is used to detect the captured IL-6. HRP-conjugated streptavidin is used to recognize the 'sandwiches'. The concentration of the IL-6 is determined by measuring the enzymatic activity of HRP using the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound HRP-streptavidin conjugate, which in turn is proportional to the concentration of the IL-6.

$$\text{Absorbance} \propto [\text{HRP-streptavidin}] \propto [\text{IL-6}]$$

A schematic description of the assay is shown in Figure 1, on page 8.

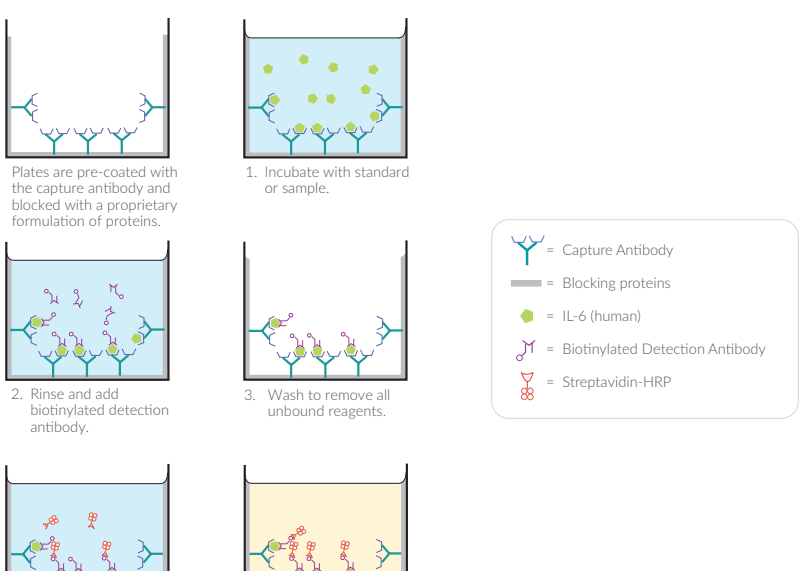


Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all buffers at 4°C; they will be stable for about two months.

1. **Assay Buffer Preparation**

   Dilute the contents of each vial of Immunoassay Buffer B Concentrate (10X) (Item No. 400054) with 90 ml of deionized water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with deionized water and add 1 ml of Polysorbate 20 (Item No. 400035).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

In general, most samples (plasma, serum, and tissue culture medium) can be assayed with no prior purification. For tissue culture medium samples, this assay performs best when the medium contains at least 0.5% carrier protein (*e.g.* BSA).

Preparation of Assay-Specific Reagents

IL-6 (human) ELISA Standard

Reconstitute the lyophilized IL-6 (human) ELISA Standard (Item No. 483364) with 2 ml of Assay Buffer. Mix gently. The concentration of this solution (the bulk standard) is 5 ng/ml. The reconstituted standard is stable for two weeks at 4°C. Enough standard is provided to produce multiple duplicate-well standard curves for use on different days, if necessary.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes or plastic microfuge tubes and label them #1 through #8. Aliquot 475 µl of Assay Buffer into tube #1, and 250 µl of Assay Buffer into tubes #2-8. Transfer 25 µl of freshly prepared stock standard (5 ng/ml) to tube #1. Mix gently. Serially dilute the standard by removing 250 µl from tube #1 and placing into tube #2. Mix gently. Next, remove 250 µl from tube #2 and place into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any IL-6 to tube #8. This tube is the zero-point, the lowest point on the standard curve.

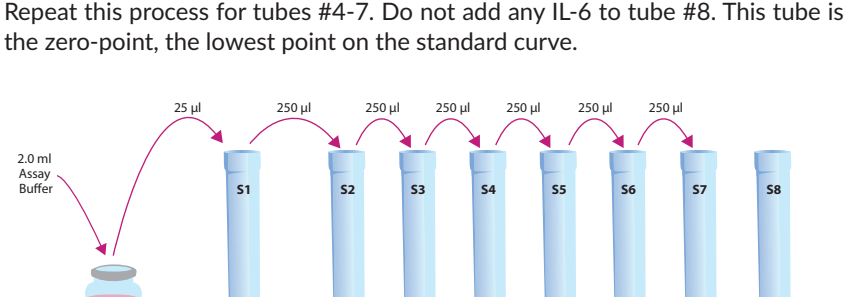


Figure 2. Preparation of the IL-6 (human) standards

Anti-IL-6 (human) Biotin Conjugate

Reconstitute the lyophilized Anti-IL-6 (human) Biotin Conjugate (Item No. 401030) with 12.0 ml of Assay Buffer. Mix gently. The reconstituted conjugate is stable for two weeks at 4°C.

IL-6 Streptavidin-HRP

This reagent is supplied as a concentrated (10X) stock solution of Streptavidin conjugated to HRP. On the day of the assay, prepare a Working Solution by adding 1.2 ml of the Streptavidin-HRP (Item No. 401033) to 10.8 ml Assay Buffer (12 ml total). This Working Solution is stable for 24 hours at 4°C, protected from light. In the event that two or more experiments are performed with this kit more than 24 hours apart, two vials of stock solution have been provided to produce an additional 12 ml of the Working Solution.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 2-4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown below in Figure 3. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see **Analysis**, page 15, for more details). We suggest you record the contents of each well on the template sheet provided (see page 21).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	
H	S8	S8	8	8	8	16	16	16	24	24	24	

S1-S8 - Standards 1-8
1-26 - Samples

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the standard, sample, and detection antibody.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of Standards and Samples and First Incubation

1. Add 100 µl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with a 96-Well Cover Sheet (Item No. 400012). Incubate for one hour at room temperature on an orbital shaker.

Addition of Anti-IL-6 (human) Biotin Conjugate and Second Incubation

1. Empty the wells and rinse five times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of the reconstituted Anti-IL-6 (human) Biotin Conjugate to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

Addition of Streptavidin-HRP and Third Incubation

1. Empty the wells and rinse five times with Wash Buffer as described above.
2. Add 100 µl of the Streptavidin-HRP Working Solution to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with Wash Buffer as described above.
2. Add 100 µl of TMB Substrate Solution (Item No. 400072) to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at room temperature in the dark. Development of the blue color can be monitored at 650 nm.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used.

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a quadratic equation. Using the equation of the line, calculate the concentration of IL-6 in each sample.

Performance Characteristics

Sensitivity:

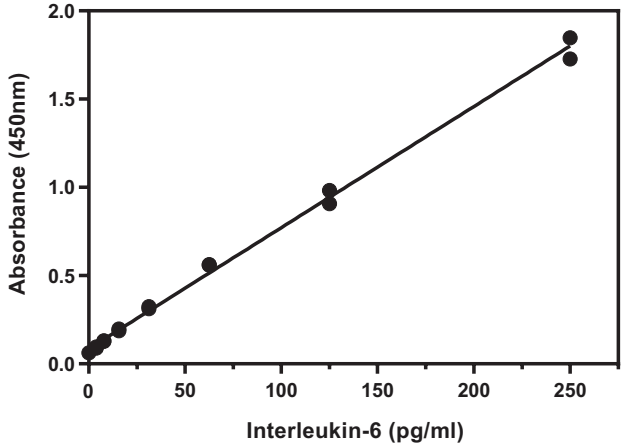
The minimum detectable concentration is 7.8 pg/ml.

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

IL-6 (pg/ml)	Absorbance	
250	1.727	1.847
125	0.907	0.982
62.5	0.562	0.559
31.2	0.312	0.325
15.6	0.198	0.188
7.8	0.131	0.127
3.9	0.095	0.089
0	0.063	0.064

Table 2. Typical results



Assay Range = 7.8-250 pg/ml
LLOQ = 7.8 pg/ml

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. – (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.).The standard was diluted with Assay Buffer.

Figure 4. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 17.

IL-6 (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
250	4.12	5.40
125	5.06	7.01
62.5	8.38	7.31
31.2	5.06	4.11
15.6	6.98	4.30
7.8	7.64	13.32
3.9	9.28	26.22

Table 3. Intra- and inter-assay variation

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of deionized water
Poor development (low signal) of standard curve	A. Standard was diluted incorrectly B. Plate requires more development time C. Standard is degraded	A. Perform assay again using correct standard dilutions B. Repeat assay with longer development time C. Obtain a new standard
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	Run standard curve in the same matrix as the samples
Sample concentrations appear inconsistent with literature values	Matrix for samples and standards are different	Use same matrix for all samples and standards

References

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3. Kishimoto, T. The biology of interleukin-6. *Blood* **74**, 1-10 (1989).
4. Muraguchi, A., Hirano, T., Tang, B., *et al.* The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. *J. Exp. Med.* **167**, 332-344 (1988).
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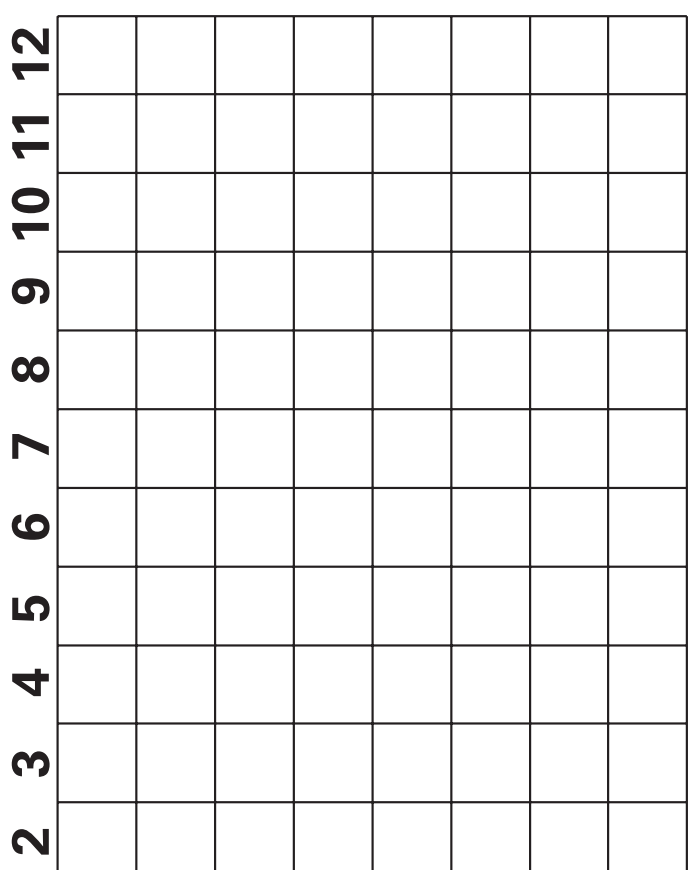

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.