Interleukin-6 (human) EIA Kit

Item No. 583361

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
483362	Anti-IL-6 (human) EIA Strip Plate	1 plate	5 plates
483360	IL-6 (human) AChE Fab' Conjugate	1 vial/100 dtn	1 vial/500 dtn
483364	IL-6 (human) EIA Standard	1 vial	1 vial
400060	EIA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400100	Human Plasma	1 vial/100 dtn	1 vial/500 dtn
400110	Non-Specific Mouse Serum	1 vial/100 dtn	1 vial/500 dtn

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

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 Chemical's ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: 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 -20°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 between 405-420 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Background

Interleukin-6 (IL-6) is a 212 amino acid polypeptide produced by various cells including T-cells, monocytes and a number of tissues. 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, 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 proliferation of other cells, including T-cells, mesangial cells, keratinocytes, Kaposi sarcoma cells, and synovial fibroblasts.[5,6]

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.[7]

About This Assay

Cayman's IL-6 (human) EIA Kit is an immunometric (*i.e.,* sandwich) EIA that permits IL-6 measurements within the range of 0-250 pg/ml, typically with a limit of detection of 7.8 pg/ml.

Description of ACE™ Immunometric EIAs

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the plate supplied with the kit has been coated with a monoclonal antibody specific for IL-6 (IL-6 capture antibody). This antibody will bind any IL-6 introduced into the well. An acetylcholinesterase:Fab' Conjugate (AChE:Fab'), which binds selectively to a different epitope on the IL-6 molecule, is also added to the well. When interleukin (standard and sample) is added to the well, the two antibodies form a 'sandwich' by binding on opposite sides of the IL-6 molecule. The 'sandwiches' are immobilized on the plate so the excess reagents may be washed away. The concentration of the analyte is then determined by measuring the enzymatic activity of the AChE by adding Ellman's Reagent (which contains the substrate for AChE) to each well. The product of the AChE-catalyzed reaction has a distinct yellow color which absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound conjugate which in turn is proportional to the concentration of the IL-6.

$$\text{Absorbance} \propto [\text{AChE:Fab' Conjugate}] \propto [\text{IL-6}]$$

A schematic description of the assay is given in Figure 1 below.

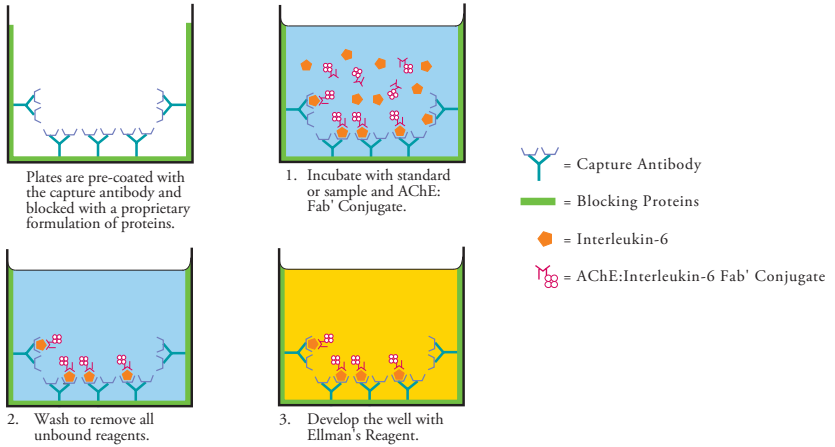

Figure 1. Schematic of the ACE™ EIA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid acetylcholinesterase (AChE) capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 s^{-1}) for the hydrolysis of acetylthiocholine.

A molecule of acetylcholinesterase covalently attached to an analyte-specific antibody serves as the conjugate in ACE™ enzyme immunometric assays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 2, on page 8). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows re-development of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.

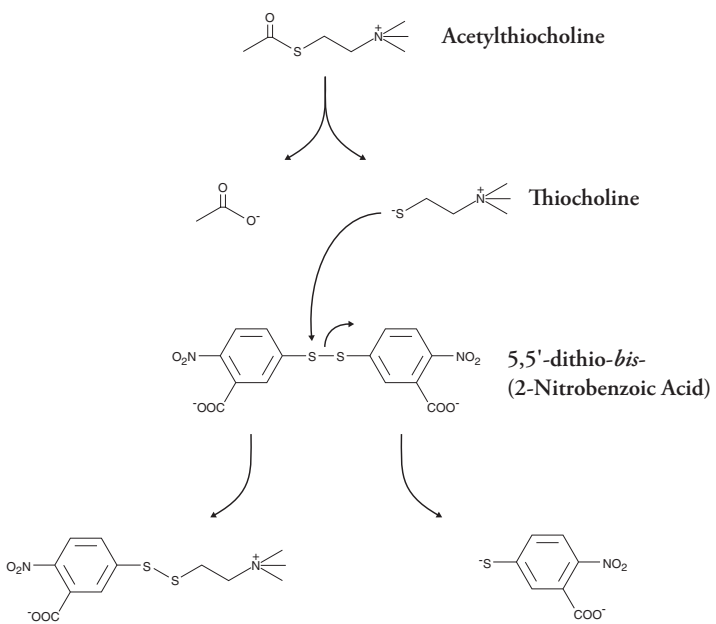

Figure 2. Reaction catalyzed by acetylcholinesterase

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. Even freshly prepared Ellman's Reagent has some measurable absorbance, approximately 0.1 Absorbance Units (A.U.). The blank absorbance should be subtracted from the absorbance readings of all the other wells.

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

Sample Matrix Blank (SMB): in order to accurately assay unpurified samples, the standards must be present in the same biological fluid ('matrix') as the samples. One must obtain a supply of this 'matrix' (plasma, synovial fluid, cell culture medium, etc.) which does not contain IL-6; this is the Sample Matrix Blank. The SMB is used as the diluent for the standard curve. *NOTE: We supply a Human Plasma SMB as part of this kit. If your samples are in any other matrix, sufficient quantities of this matrix must be obtained to use as the diluent for your standard curve.*

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

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

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

1. EIA Buffer Preparation

Dilute the contents of one vial of EIA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure 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 Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035).

OR

12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062): Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Polysorbate 20 (Item No. 400035).

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer).

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, samples can be assayed with no prior purification. If human plasma or synovial fluid is to be tested, one must add the non-specific mouse immunoglobulin (supplied in the kit) to each sample and each point of the standard curve. This will compensate for the effects of human anti-mouse IgG which may be present in the samples. Samples of synovial fluid from patients with rheumatoid arthritis often contain rheumatoid factors (IgM anti-Fc) which can bind mouse IgG and cause erroneously high values. The addition of both the non-specific mouse serum and dithiothreitol (DTT) will alleviate this problem.[8] Remember: the standard curve wells must contain the same 'matrix' (including mouse serum and DTT) as the sample wells. Plasma and serum samples should be assayed at a 1:2 dilution with EIA Buffer.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Preparation of Assay-Specific Reagents

Sample Matrix Blank (SMB) - Human Plasma

The SMB provided (Item No. 400100) with this kit is IL-6-free human plasma. If this matches your samples, reconstitute with 5 ml (100 dtn) UltraPure water or 25 ml (500 dtn). Store this solution at 4°C; it will be stable for approximately two weeks. If your SMB is not human plasma, you must obtain an IL-6 free SMB that matches your samples. *NOTE: The SMB provided is enough to run one standard curve once reconstituted. More SMB may be purchased by ordering Cayman Human Plasma (Item No. 400100).*

IL-6 (human) EIA Standard

Dilute the IL-6 (human) EIA Standard (Item No. 483364) with 2 ml EIA Buffer. The concentration of this solution will be 5 ng/ml (5,000 pg/ml). Store this solution at 4°C; it will be stable for approximately two weeks. We have included enough IL-6 to run ten standard curves. This surplus should accommodate any experimental design.

NOTE: If assaying culture medium samples that have not been diluted with EIA Buffer, culture medium should be used in place of EIA Buffer for dilution of the standard curve.

To prepare the standard for use in EIA: Obtain eight clean test tubes and label them #1 through #8. Aliquot 950 µl SMB to tube #1 and 500 µl SMB to tubes #2-8. Transfer 50 µl of the bulk standard (5 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process until tube #7 has been prepared. Do not add any IL-6 to tube #8. This tube is the zero-point vial, the lowest point on the standard curve. These diluted standards should not be stored for more than 24 hours.

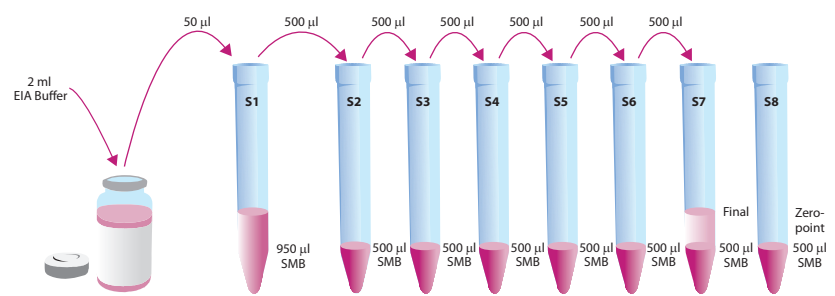

Figure 3. Preparation of the IL-6 standards

Non-specific Mouse Serum

The mouse serum supplied with this kit is to be used when analyzing unpurified plasma, serum, synovial fluid or any other sample that may contain heterophilic antibodies.[9] Reconstitute the mouse serum (Item No. 400110) with 2.5 ml (100 dtn) or 12.5 ml (500 dtn) UltraPure water and store at 4°C. It will be stable for approximately two weeks. A 25 µl aliquot of the mouse serum should be added to each 500 µl aliquot of sample or standard prior to addition to the well. Remember, you must also add the mouse serum to each point of the standard curve (25 µl of mouse serum per 500 µl of standard) to ensure a uniform SMB.

Dithiothreitol - Not included in this kit

If you suspect your samples contain anti-mouse IgM (rheumatoid factors), add 50 µl of 100 mM DTT to a 500 µl aliquot of each sample. Remember, you must also add the DTT to each point of the standard curve (50 µl of DTT per 500 µl of standard) to ensure a uniform SMB. *NOTE: This is a comparative assay. Although the addition of mouse serum and DTT will change the concentration of your samples and standards, the change will be proportional throughout the assay.*

IL-6 (human) AChE Fab' Conjugate

Reconstitute the Fab' Conjugate as follows:

100 dtn IL-6 (human) AChE Fab' Conjugate (96-well kit; Item No. 483360): Reconstitute with 10 ml EIA Buffer.

OR

500 dtn IL-6 (human) AChE Fab' Conjugate (480-well kit; Item No. 483360): Reconstitute with 50 ml EIA Buffer.

Store the reconstituted Fab' Conjugate at 4°C *(do not freeze!)* and use within two weeks. A 10% surplus of Fab' Conjugate has been included to account for any incidental losses.

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 without rinsing and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and 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 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 in Figure 4, on page 15. 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 19, for more details). We suggest you record the contents of each well on the template sheet provided (see page 27).

	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	Blk
H	S8	S8	8	8	8	16	16	16	24	24	24	Blk

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

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- 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 the Reagents

1. IL-6 (human) EIA Standard

Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

2. Samples

Add 100 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

3. IL-6 (human) AChE Fab' Conjugate

Add 100 µl to each well *except* the Blank (Blk) wells.

Well	Standard	Sample	AChE:IL-6 Conjugate
Blk	-	-	-
Standard	100 µl	-	100 µl
Sample	-	100 µl	100 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate overnight at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   100 dtn vial Ellman's Reagent (96-well kit; Item No. 400050): Reconstitute with 20 ml of UltraPure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050): Reconstitute with 50 ml of UltraPure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays be run on different days.

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well
4. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark.
5. The absorbance of the wells can be checked periodically over the next few hours.

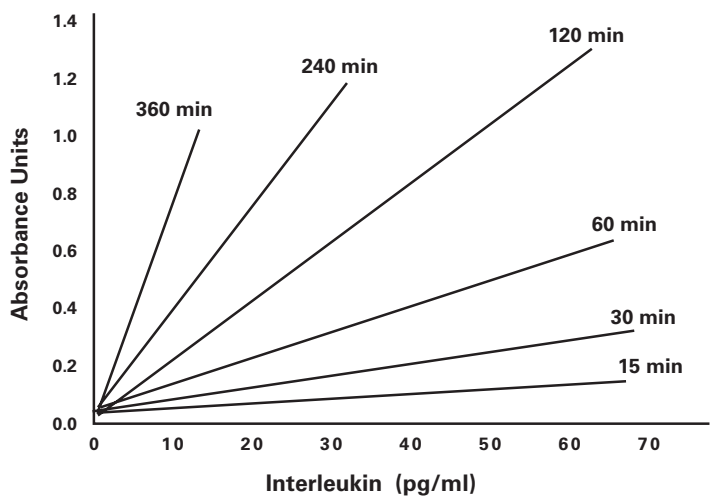

Figure 5. Standard curve at various development times

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*
3. Read the plate at a wavelength between 405-420 nm. Once the S1 wells seem visibly yellow (0.3 A.U., ~60 minutes) it will be possible to determine the concentration of the relatively concentrated samples. Longer development times will be necessary to obtain an accurate plot for the lower range of the standard curve and statistically significant values for sample concentrations near the detection limit of the assay (~1.5 pg/ml). Standard curves at various development times are shown above in Figure 5.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. *NOTE: Cayman Chemical has a computer spreadsheet available for data anaylsis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

1. If your plate reader has not already done so, subtract the average absorbance of the blank wells from the absorbance readings for the rest of the plate.
2. Calculate the average absorbance for each standard and sample well.

Plot the Standard Curve

Plot absorbance *versus* concentration for standards S1-S8. Construct a best-fit line through the points, including the S8 point.

Determine the Sample Concentration

Use the equation of the line to calculate the concentration of your samples.

$$\text{IL-6 Concentration (pg/ml)} = \left[\frac{(A_{412} \text{ sample}) - (\text{Y-intercept})}{\text{Slope}} \right] \times \text{Dilution}$$

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. Your results could differ substantially.

IL-6 (pg/ml)	Absorbance	
250	0.973	0.983
125	0.528	0.524
62.5	0.280	0.279
31.3	0.129	0.128
15.6	0.075	0.076
7.8	0.044	0.043
3.9	0.025	0.026
0	0.022	0.020

Table 2. Typical results

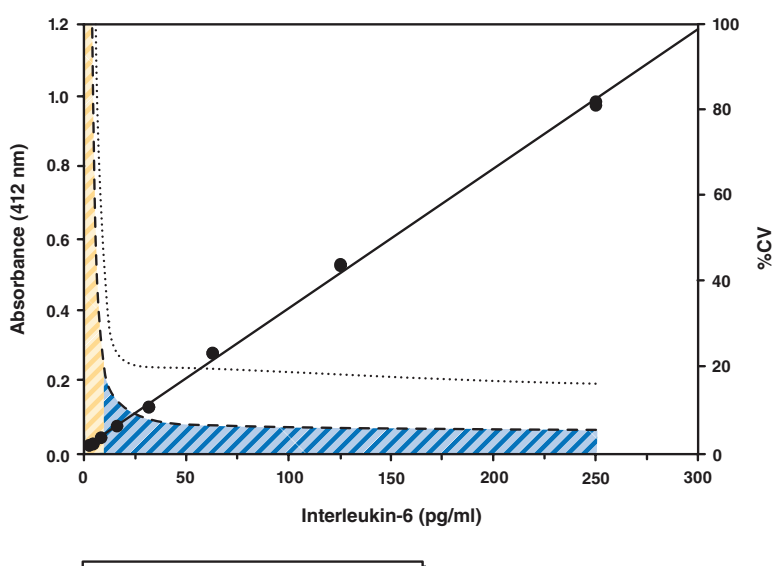

Figure 6. Typical standard curve

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 21 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
250	4.3	15.6
125	4.7	16.7
62.5	6.6	22.2
31.3	7.9	27.8
15.6	14.9	23.1
7.8	23.4	†
3.9	†	†
0	†	†

Table 3. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

Specificity:

Compound	Cross Reactivity
Interleukin-6	100%
Granulocyte Colony-Stimulating Factor	<0.01%
Macrophage Colony-Stimulating Factor	<0.01%
Interleukin-1α	<0.01%
Interleukin-1β	<0.01%
Interleukin-2	<0.01%
TNF-α	<0.01%

Table 4. Specificity of the IL-6 assay

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 UltraPure water
Poor development (low signal) of standard curve.	A. Standard was diluted incorrectly B. Standard is degraded C. Plate requires more development time	A. Replace activated carbon filter or change source of UltraPure water B. Return plate to shaker and re-read later
Analyses of two dilutions of a biological sample do not agree (*i.e.,* more than 20% difference).	Interfering substances are present	A. Sample must be purified prior to analysis by EIA.[9] B. Add mouse serum and DTT to standards and samples.
Sample concentrations appear inconsistent with literature values.	Matrix for samples and standards are different	A. Use same matrix for all samples and standards B. Add mouse serum and DTT to standards and samples.

References

1. Van Damme, J., Opdenakker, G., Simpson, R.J., *et al.* Identification of the human 26-kD protein, interferon β_2 (IFN-β_2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* **165**, 914-919 (1987).
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3. Kishimoto, T. The biology of interleukin-6. *Blood* **74**, 1-10 (1989).
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5. Tosato, G., Seamon, K.B., Goldman, N.D., *et al.* Monocyte-derived human B-cell growth factor identified as interferon-β_2 (BSF-2, IL-6). *Science* **239**, 502-504 (1988).
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7. Hirano, T., Akira, S., Taga, T., *et al.* Biological and clinical aspects of interleukin 6. *Immunol. Today* **11**, 443-449 (1990).
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Related Products

Interleukin-1α (human) EIA Kit - Item No. 583301
Interleukin-1β (human) EIA Kit - Item No. 583311
Interleukin-2 (human) EIA Kit - Item No. 583321
Interleukin-4 (human) EIA Kit - Item No. 583341
Interleukin-4 (mouse) EIA Kit - Item No. 500860
Interleukin-5 (mouse) EIA Kit - Item No. 500880
Interleukin-6 (mouse) EIA Kit - Item No. 583371
Interleukin-13 (mouse) EIA Kit - Item No. 500870
Interleukin-17A (mouse) EIA Kit - Item No. 500970
Luminex® Prostaglandin E2/Interleukin-1β Duplex Kit - Item No. 10009597
TNF-α (human) EIA Kit - Item No. 589201
TNF-α (mouse) EIA Kit - Item No. 500850
UltraPure Water - Item No. 400000

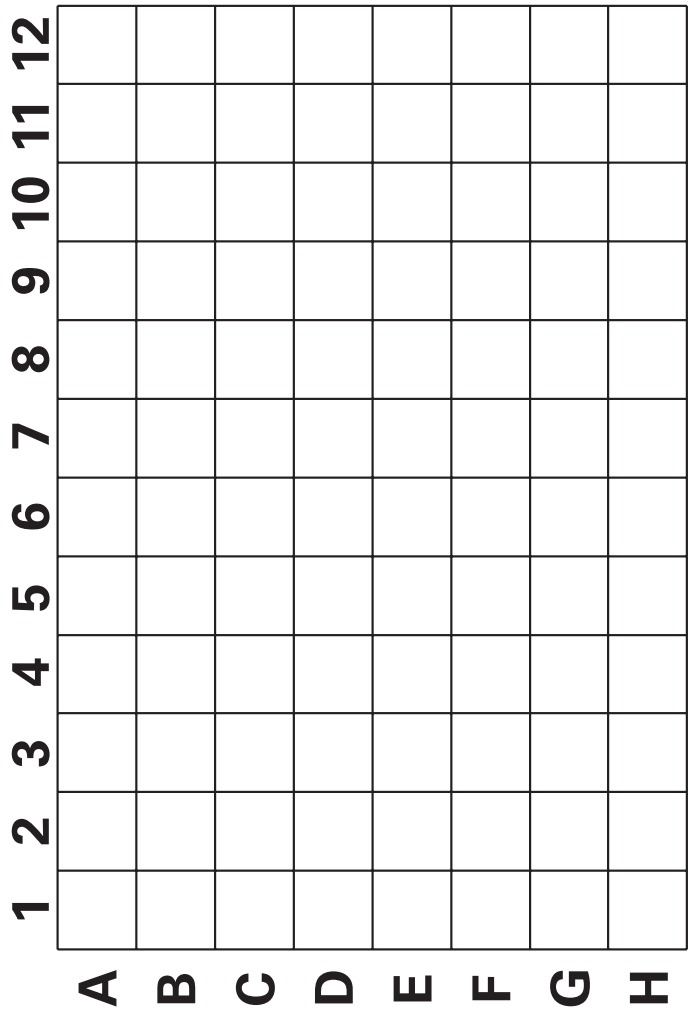
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES