Interleukin-1β (human) ELISA Kit

Item No. 583311

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
483312	Anti-Interleukin-1β (human) ELISA Strip Plate	1 plate	4°C
483310	Interleukin-1β (human) AChE Fab' Conjugate	1 vial/100 dtn	-20°C
483314	Interleukin-1β (human) ELISA Standard	1 vial	-20°C
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400012	96-Well Cover Sheet	1 cover	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400100	Human Plasma	1 vial/100 dtn	-20°C
400110	Non-Specific Mouse Serum	1 vial/100 dtn	-20°C

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 Chemical's Interleukin-1β (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

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

Email: techserv@caymanchem.com

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 repeating pipettor.
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 13).

INTRODUCTION

Background

Interleukin-1 (IL-1) is a name for a family of proteins which includes IL-1α, IL-1β, Interleukin receptor antagonist (ILra) and IL-18. IL-1β is synthesized as 269 amino acid pro-cytokine that is enzymatically cleaved by IL-1β-converting enzyme (ICE) into the active 154 amino acid, 17 kDa mature peptide.[1,2] IL-1β is produced by many cell types including macrophages, monocytes, astrocytes, endothelial cells, smooth muscle cells, keratinocytes, adipocytes, T cells, and eosinophils.[3] Although IL-1α and IL-1β exhibit only 26% amino acid identity,[1] they bind to the same cell-surface receptors, IL-1 RI and IL-1 RII, present on a variety of cell types involved in immune or inflammatory responses.[4-6] Normal production of IL-1 is critical for mediating host responses to infection and injury.[2,3] Disease states in which IL-1 actively participates include inflammatory diseases, such as arthritis, inflammatory bowel disease, and shock, as well as atherosclerosis, allergic disease, and some types of cancer.[3,7]

About This Assay

Cayman's IL-1β (human) ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA that permits IL-1β measurements within the range of 3.9-250 pg/ml, typically with a limit of detection of approximately 3.9 pg/ml.

Description of AChE 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 monoclonal antibody specific for IL-1β (IL-1β capture antibody). This antibody will bind any IL-1β introduced into the well. An acetylcholinesterase:Fab' Conjugate (AChE:Fab'), which binds selectively to a different epitope on the IL-1β molecule, is also added to the well. This allows the two antibodies to form a 'sandwich' by binding on opposite sides of the IL-1β 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-1β.

$$\text{Absorbance} \propto [\text{AChE:Fab' Conjugate}] \propto [\text{IL-1}\beta]$$

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

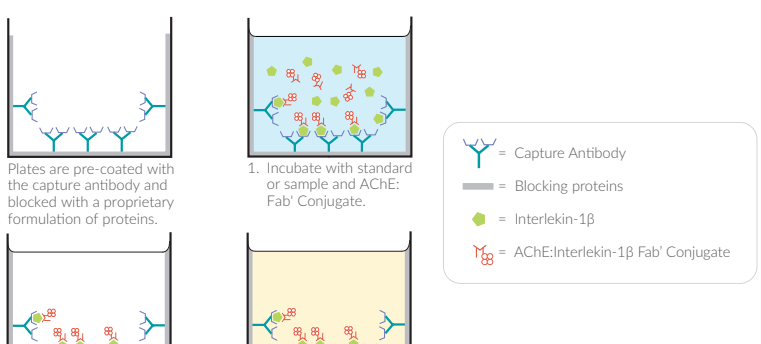

Figure 1. Schematic of the AChE ELISA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *E. electricus*, contains an avid 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 AChE 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 10). 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 redevelopment 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.

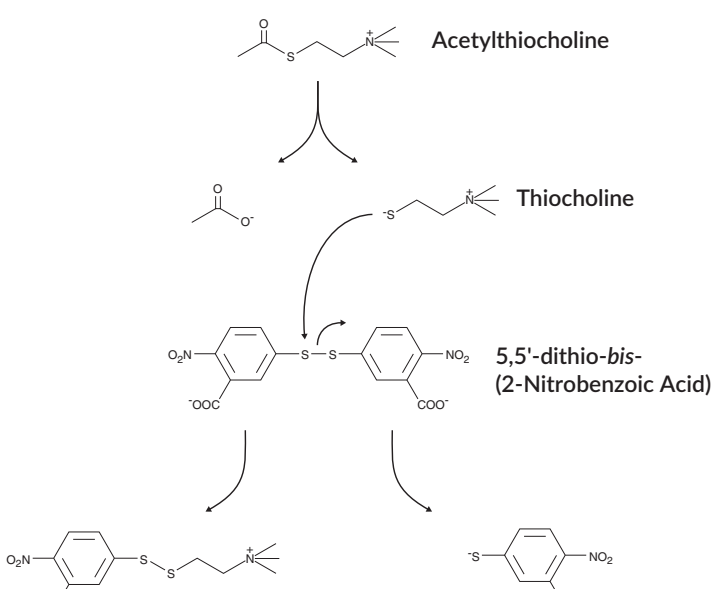

Figure 2. Reaction catalyzed by acetylcholinesterase

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-1β; 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.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all ELISA 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 ELISA. 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. **ELISA Buffer Preparation**

   Dilute the contents of one vial of ELISA 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**

   Dilute to a total volume of 2 liters with UltraPure water and add 1 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 anti-mouse IgM (rheumatoid factors) which can cause erroneously high values. The addition of both the Non-specific Mouse Serum and dithiothreitol (DTT) will alleviate this problem.[11] Remember: The standard curve wells must contain the same 'matrix' (including Mouse Serum and DTT) as the sample wells.

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 Human Plasma SMB (Item No. 400100) provided with this kit is IL-1β-free human plasma. If this matches your samples, reconstitute with 5 ml of UltraPure water. 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-1β-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's Human Plasma (Item No. 400100).*

IL-1β (human) ELISA Standard

Reconstitute the IL-1β (human) ELISA Standard (Item No. 483314) with 2 ml ELISA 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-1β to run twenty standard curves. This surplus should accommodate any experimental design.

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

Obtain eight clean test tubes and label them #1 through #8. Aliquot 50 µl from the bulk standard (5,000 pg/ml) into tube #1 and dilute to 1.0 ml with SMB and vortex briefly. The IL-1β concentration of this tube is now 250 pg/ml, and is the highest point on the standard curve. Next add 500 µl of SMB to tubes #2 through #8. Aliquot 500 µl from tube #1 into tube #2 and vortex briefly. The concentration of tube #2 will be 125 pg/ml. Add 500 µl from tube #2 to tube #3 and vortex briefly. The IL-1β concentration of tube #3 will now be 62.5 pg/ml. Continue this procedure until tube #7 has been prepared (it will be 3.9 pg/ml). Do not add any IL-1β 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.

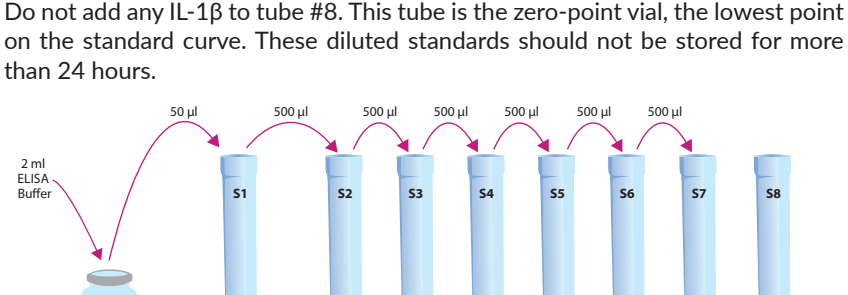

Figure 3. Preparation of the IL-1β standards

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.[12] Reconstitute the Non-specific Mouse Serum (Item No. 400110) with 2.5 ml 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-1β (human) AChE Fab' Conjugate

Reconstitute with 10 ml ELISA 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 below in Figure 4. 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 23, for more details). We suggest you record the contents of each well on the template sheet provided (see page 30).

	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-1β (human) ELISA 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-1β (human) AChE Fab' Conjugate**

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

Well	Standard	Sample	AChE:IL-1β Conjugate
Blk	-	-	-
Standard	100 µl	-	100 µl
Sample	-	100 µl	100 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with 96-Well Cover Sheet (Item No. 400012) and incubate overnight at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use with 20 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.

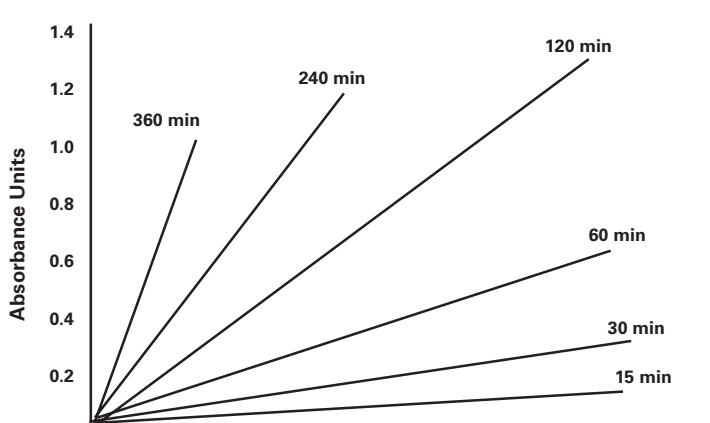

Figure 5. Standard curve at various development times

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., ~20-30 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 analysis. 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.

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

Performance Characteristics

Sensitivity:

The minimum detectable concentration is 3.9 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-1β (pg/ml)	Absorbance	
250	1.360	1.375
125	0.733	0.736
62.5	0.406	0.397
31.3	0.222	0.228
15.6	0.135	0.135
7.8	0.088	0.092
3.9	0.064	0.069
0	0.036	0.040

Table 2. Typical results

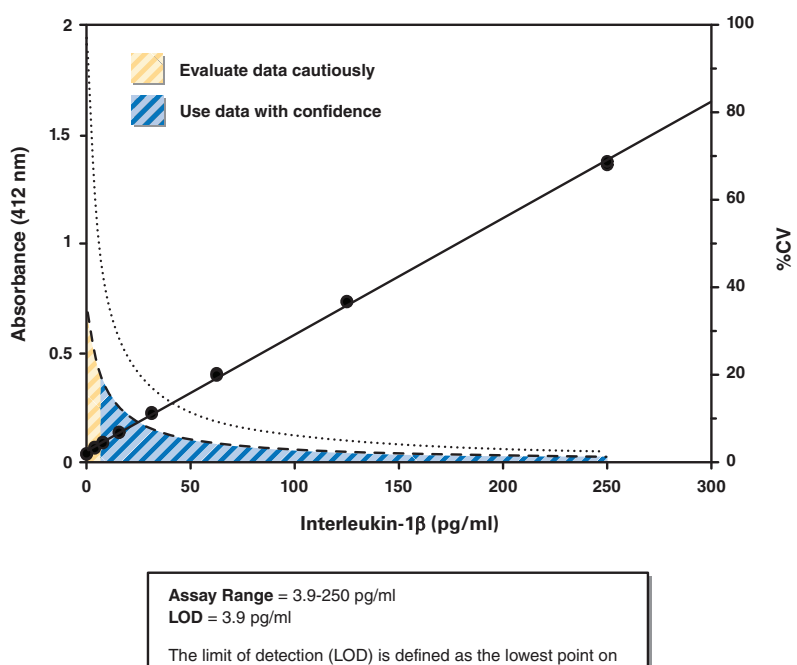

Figure 6. Typical standard curve

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 25 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
250	1.6	2.3
125	2.0	3.8
62.5	2.4	7.9
31.3	4.2	7.5
15.6	8.1	11.4
7.8	12.7	†
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.

Cross Reactivity:

Compound	Cross Reactivity
Interleukin-1β	100%
Interleukin-1α	<0.01%
Interleukin-2	<0.01%

Table 4. Cross Reactivity of the IL-1β (human) 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. Plate requires more development time B. Standard was diluted incorrectly C. Standard is degraded	A. 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 ELISA[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

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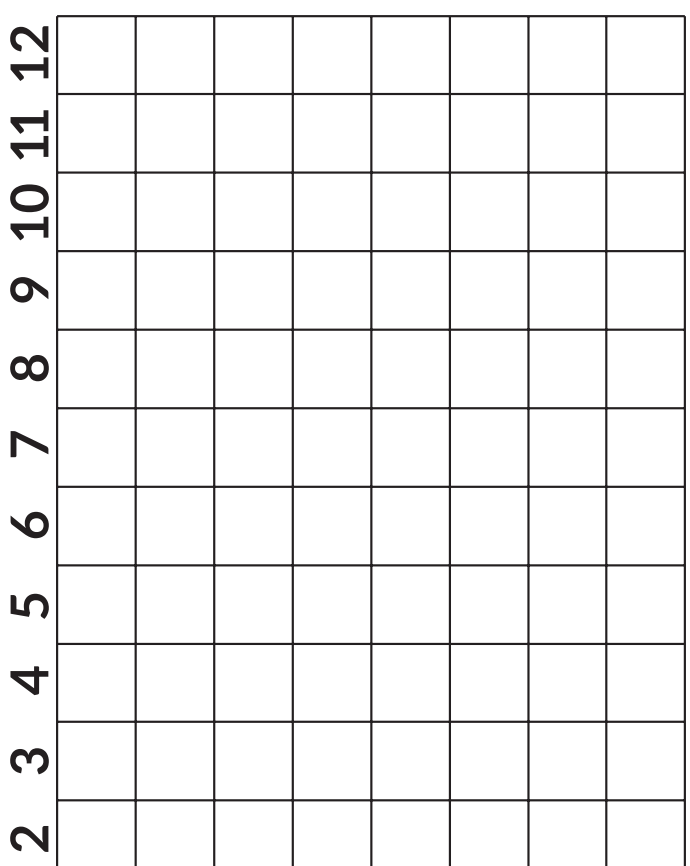

NOTES

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.

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