



Catalog # KMC0011 (96 tests) KMC0012 (192 tests) KMC0011C (480 tests)

Mouse IL-1β

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Contents and Storage

Storage Store at 2 to 8°C.

Contents

| | 96 | 192 | 480 |
|--|----------|-----------|-----------|
| Reagent | Test Kit | Test Kit | Test Kit |
| Ms IL-1ß Standard, recombinant Ms IL-1ß. Contains 0.1% | 2 vials | 4 vials | 10 vials |
| sodium azide. Refer to vial label for quantity and reconstitution volume. | | | |
| <i>Standard Diluent</i> Buffer. Contains 0.1% sodium azide; 25 mL per bottle. | 1 bottle | 2 bottles | 5 bottles |
| Incubation Buffer. 12 mL per bottle. | 1 bottle | 1 bottle | 5 bottles |
| Antibody Coated Wells. 12 x 8 Well Strips. | 1 plate | 2 plates | 5 plates |
| MsIL-1BBiotinConjugate(Biotin-labeledanti-MsIL-1B).Contains0.1%sodium azide;6 mL per bottle. | 1 bottle | 2 bottles | 5 bottles |
| <i>Streptavidin-HRP (100X).</i> Contains 3.3 mM thymol; 0.125 mL per vial. | 1 vial | 2 vials | 5 vials |
| <i>Streptavidin-HRP Diluent.</i> Contains 3.3 mM thymol; 25 mL per bottle. | 1 bottle | 1 bottle | 3 bottles |
| Wash Buffer Concentrate (25X). 100 mL per bottle. | 1 bottle | 1 bottle | 2 bottles |
| <i>Stabilized Chromogen, Tetramethylbenzidine (TMB).</i> 25 mL per bottle. | 1 bottle | 1 bottle | 3 bottles |
| Stop Solution. 25 mL per bottle. | 1 bottle | 1 bottle | 3 bottles |
| Plate Covers, adhesive strips. | 3 | 4 | 15 |

- **Disposal Note Note Note Note Note This kit contains materials with small quantities of sodium azide. Sodium azide note Note**
- **Safety** All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Purpose The Invitrogen Mouse Interleukin-1ß (Ms IL-1ß) ELISA is to be used for the quantitative determination of Ms IL-1ß in mouse serum, plasma (EDTA), buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-1ß.

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Principle of the Method Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Ms IL-1ß has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms IL-1ß content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the Ms IL-1ß antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Ms IL-1ß present in the original specimen.

Background Information Mouse IL-1ß, also known as lymphocyte activating factor, is a 17.5 kilodalton protein which contains 159 amino acids. Primary cellular sources of IL-1ß are macrophages and mononcytes, but IL-1ß is also produced by lymphocytes, epithelial cells, keratinocytes and mesenchymal cells. There is a 68% amino acid homology between mouse and human forms of IL-1ß. IL-1ß is best known for mediating the "acute phase" of the inflammatory response. IL-1ß mimics shock, is a potent pyrogen, increases the synthesis of normal and pathologic hepatic proteins, induces hypoglycemia, contributes to a negative nitrogen balance, inhibits smooth muscle contraction, and increases the production of several neuropeptides including ACTH, endorphins, vasopressin, and somatostatin (1). IL-1ß acts to stabilize mRNA for GM-CSF and induces new transcription of stem cell factors.

- **Materials** Microtiter plate reader (at or near 450 nm) with software Calibrated adjustable precision pipettes Needed • Distilled or deionized water **But Not** Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.) Provided Plastic tubes for diluting solutions Absorbent paper towels Calibrated beakers and graduated cylinders 37°C Incubator **Procedural** 1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use. Notes 2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity. Samples should be collected in pyrogen/endotoxin-free tubes. 4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis. 5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis. 6. It is recommended that all standards, controls and samples be run in duplicate. 7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells. 8. Do not mix or interchange different reagent lots from various kit lots. 9. Do not use reagents after the kit expiration date. 10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark. 11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect. 12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. *Never* insert absorbent paper directly into the wells. 13. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop. Directions • Incomplete washing will adversely affect the test outcome. All washing must be performed with the Wash Buffer Concentrate (25X) provided. for Washing
 - Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
 - If using an automated washer, follow the washing instructions carefully.

Dilution of One nanogram of Invitrogen recombinant Ms IL-1ß equals 2600 units of WHO reference preparation 93/668 (NIBSC, Hertfordshire, UK, EN6 3QG).

Note: Do not use glass tubes for the serial dilution. Use non-glass microcentrifuge or titer tubes.

1. The standard should be reconstituted to 1000 pg/mL with **either** *Standard Diluent Buffer* **or** *Tissue Culture Medium* according to the following sample type:

For serum or plasma samples: Reconstitute the standard using *Standard Diluent Buffer*. Serum or plasma samples should be quantified against a standard curve that is reconstituted and serially diluted in *Standard Diluent Buffer*.

For tissue culture supernatant samples: Reconstitute the standard using the *Tissue Culture Medium* used to culture samples. Tissue culture samples should be quantified against a standard curve reconstituted and serially diluted in the *Tissue Culture Medium* used to culture samples.

- 1. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 1,000 pg/mL Ms IL-1ß. Use the standard within 1 hour of reconstitution.
- 2. Add 0.250 mL of *Standard Diluent Buffer* (for serum and plasma samples) or *Tissue Culture Medium* (for tissue culture samples) to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL.
- 3. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Remaining reconstituted standard should be discarded. Return the *Standard Diluent Buffer* to the refrigerator.



Preparing SAV-HRP Note: Prepare within 15 minutes of usage. The *Streptavidin-HRP (100X)* is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

- 1. Dilute 10 μL of this 100X concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
- Volume of Streptavidin-HRP Volume of Diluent # of 8-Well Strips (100X) 20 µL solution 2 2 mL 4 40 µL solution 4 mL 6 60 µL solution 6 mL 8 80 µL solution 8 mL 10 100 µL solution 10 mL 12 120 µL solution 12 mL
- 2. Return the unused Streptavidin-HRP (100X) to the refrigerator.

Dilution of Wash Buffer
1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Be sure to read the *Procedural Notes* section before carrying out the assay.

Procedure Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

- 1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
- 2. Add 50 µL of the *Incubation Buffer* to all wells. Well(s) reserved for chromogen blank should be left empty.
- 3. Add 50 μL of the *Standard Diluent Buffer* (for serum and plasma samples) or *Tissue Culture Medium* (for tissue culture samples) to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
- 4. Add 50 µL of standards, controls or samples to the appropriate microtiter wells. (See **Preparation of Reagents**.) Tap gently on side of plate to mix.
- 5. Pipette 50 µL of biotinylated *Ms IL-1ß Biotin Conjugate* solution into each well except the chromogen blank(s). Tap on the side of the plate to thoroughly mix.
- 6. Cover plate with *plate cover* and incubate for **1 hour and 30 minutes at 37°C**.
- 7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
- 8. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
- 9. Cover plate with the *plate cover* and incubate for **30 minutes at room**

temperature.

- 10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions fro Washing**.
- 11. Add 100 μL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
- 12. Incubate for **30 minutes at room temperature and in the dark**. *Note:* **Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
- 13. Add 100 μL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
- 14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
- 15. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
- 16. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be further diluted in *Standard Diluent Buffer* (serum of plasma) or *Tissue Culture Medium* (tissue culture samples) and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TypicalThe following data were obtained for the various standards over the range of 0 to
1,000 pg/mL Ms IL-1ß.

(Example)

| Standard | Optical Density |
|------------------|-----------------|
| Ms IL-1ß (pg/mL) | (450 nm) |
| | |
| 1,000 | 2.85 |
| | |
| 500 | 1.71 |
| | |
| 250 | 0.964 |
| | |
| 125 | 0.56 |
| | |
| 62.5 | 0.34 |
| | |
| 31.2 | 0.229 |
| | |
| 15.6 | 0.198 |
| | |
| 0 | 0.117 |

Sensitivity The minimum detectable dose of Ms IL-1ß is <7 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision 1. Intra-Assay Precision

Samples of known Ms IL-1ß concentration were assayed in replicates of 16 to determine precision within an assay.

| - | Sample 1 Sam | | Sample 3 | | |
|--|-----------------|-------|----------|--|--|
| Mean (pg/mL) | 188.1 | 435.0 | 755.6 | | |
| SD | 8.9 | 23.8 | 33.4 | | |
| %CV | 6CV 4.7 5.5 4.4 | | 4.4 | | |
| SD = Standard Deviation CV = Coefficient of Variation | | | | | |

2. Inter-Assay Precision

Samples were assayed 30 times in multiple assays to determine precision between assays.

| | Sample 1 | Sample 2 | Sample 3 | | |
|--|----------|----------|----------|--|--|
| Mean (pg/mL) | 201.9 | 400.0 | 825.8 | | |
| SD | 17.9 | 29.9 | 75.4 | | |
| %CV | 8.8 | 7.4 | 9.1 | | |
| SD = Standard Deviation CV = Coefficient of Variation | | | | | |

Linearity of Dilution Mouse serum and tissue culture medium containing 10% fetal bovine serum were spiked with Ms IL-1ß and serially diluted over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

| | Serum | | Cell Culture | | | |
|----------|----------|----------|--------------|----------|----------|----------|
| | Measured | Expected | % | Measured | Expected | % |
| Dilution | (pg/mL) | (pg/mL) | Expected | (pg/mL) | (pg/mL) | Expected |
| neat | 641 | - | - | 493 | - | - |
| 1/2 | 337 | 321 | 105 | 245 | 242 | 99 |
| 1/4 | 183 | 160 | 114 | 130 | 121 | 106 |
| 1/8 | 91 | 80 | 114 | 54 | 60 | 87 |
| 1/16 | 45 | 40 | 113 | 31 | 30 | 101 |

Recovery The recovery of Ms IL-1ß added to mouse serum averaged 90% The recovery of Ms IL-1ß added to plasma (EDTA) averaged 97%. The recovery of Ms IL-1ß added to tissue culture medium containing 1% fetal bovine serum averaged 101%, while the recovery of Ms IL-1ß added to tissue culture medium containing 10% fetal bovine serum averaged 105%.

Specificity Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Invitrogen Ms IL-1ß kit. The following substances were tested and found to have no cross-reactivity: human IL-1ß, IL-2, IL-4, IL-6, IL-7, IL-10, IFN- γ , TNF- α ; mouse IL-1 α IL-2, IL-3, IL-4, IL-6, IL-10, IFN- γ , TNF- α ; rat IL-1 α , IFN- γ ; swine IL-1ß and rat IL-1ß showed 0.3% and 12% cross-reactivity, respectively.

High Dose A sample spiked with Ms IL-1ß up to 39 ng/mL gave a response higher than that obtained for the last standard point.

Expected Values Ten sera and ten plasma (EDTA) samples were evaluated in this assay. The values for sera ranged from 0 to 18 pg/mL (mean = 6.6 pg/mL). The values for plasma ranged from 0 to 19 pg/mL (mean = 6.8 pg/mL).

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for released Ms IL-1ß.

LPS (25 mg/mL), PHA (5 mg/mL) 4 hr: 17 pg/mL LPS (25 mg/mL), PHA (5 mg/mL) 24 hr: 41 pg/mL Con-A (5 mg/mL) 6 hr: 0 pg/mL PMA (50 ng/mL), Ionophore (250 ng/mL) 12 hr: 61 pg/mL

Limitations of the **Procedure** Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*, reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Ms IL-1ß in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Troubleshooting Guide

| Elevated background | <i>Cause:</i> Insufficient washing and/or draining of wells after washing. Solution containing either biotin or Streptavidin-HRP can elevate the background if residual is left in the well. |
|---------------------------------|--|
| | washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain and tap forcefully if necessary to remove residual fluid. |
| | <i>Cause:</i> Contamination of substrate solution with metal ions or oxidizing reagents. <i>Solution:</i> Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. |
| | <i>Cause:</i> Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate. <i>Solution:</i> Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. |
| | <i>Cause:</i> Incubation time is too long or incubation temperature is too high. <i>Solution:</i> Reduce incubation time and/or temperature. |
| Elevated sample/ standard | <i>Cause:</i> Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. <i>Solution</i> : Follow the protocol instructions regarding the dilution of the standard. |
| ODs | <i>Cause:</i> Incorrect dilution of the Streptavidin-HRP Working Solution. <i>Solution:</i> Warm solution of Streptavidin-HRP (100X) to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in Streptavidin-HRP Diluent provided. |
| | <i>Cause</i> : Incubation times extended. Solution: Follow incubation times outlined in protocol. |
| | <i>Cause:</i> Incubations carried out at 37°C when RT is dictated. <i>Solution:</i> Perform incubations at RT (= 25 ± 2 °C) when instructed in the protocol. |
| Poor standard curve | <i>Cause:</i> Improper preparation of standard stock solution. <i>Solution:</i> Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample. |
| | <i>Cause:</i> Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. <i>Solution:</i> NEVER substitute any components from another kit. |
| | <i>Cause</i> : Errors in pipetting the standard or subsequent steps. <i>Solution</i> : Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. |

| Weak/no color | Cause: Reagents not at RT (25 \pm 2°C) at start of assay. Solution: Allow ALL reagents to warm to RT prior to commencing assay. |
|-------------------|--|
| develops | <i>Cause:</i> Incorrect storage of components, e.g., not stored at 2 to 8°C. <i>Solution:</i> Store all components exactly as directed in protocol and on labels. |
| | <i>Cause:</i> Working Streptavidin-HRP solution made up longer than 15 minutes before use in assay. |
| | Solution: Use the diluted Streptavidin-HRP within 15 minutes of dilution. |
| | <i>Cause:</i> TMB solution lost activity. <i>Solution 1:</i> The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. <i>Solution 2:</i> Avoid contact of the TMB solution with items containing metal ions. |
| | <i>Cause:</i> Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. <i>Solution:</i> Please contact Technical Support for advice when using nonvalidated sample types. |
| | <i>Cause:</i> Wells have been scratched with pipette tip or washing tips. <i>Solution:</i> Use caution when dispensing and aspirating into and out of microwells. |
| Poor Precision | <i>Cause:</i> Errors in pipetting the standards, samples or subsequent steps. <i>Solution:</i> Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. |
| | <i>Cause:</i> Repetitive use of tips for several samples or different reagents. <i>Solution:</i> Use fresh tips for each sample or reagent transfer. |
| | <i>Cause:</i> Wells have been scratched with pipette tip or washing tips. <i>Solution:</i> Use caution when dispensing and aspirating into and out of microwells. |

Technical Support

Contact Us For more troubleshooting tips, information, or assistance, please call, email, or go online to <u>www.invitrogen.com/ELISA</u>.



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Explanation of symbols

| Symbol | Description | Symbol | Description |
|----------------|------------------------------|-------------|---|
| ** | Manufacturer | REF | Catalog number |
| | Use by | X | Temperature limitation |
| [<u>```</u>] | Consult instructions for use | \triangle | Caution, consult accompanying documents |
| LOT | Batch code | | |

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Mouse IL-1 β Assay Summary

