ELISA Kit

Catalog #
KMC0021 (96 tests)
KMC0022 (192 tests)
KMC0021C (480 tests)

New and Improved Kit
Please read protocol carefully.
To view changes, see page 18.

Mouse
IL-2

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Contents and Storage

Storage
Store at 2 to 8°C.

Contents

<table>
<thead>
<tr>
<th>Reagents Provided</th>
<th>96 Test Kit</th>
<th>192 Test Kit</th>
<th>480 Test Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms IL-2 Standard, recombinant Ms IL-2. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.</td>
<td>2 vials</td>
<td>4 vials</td>
<td>10 vials</td>
</tr>
<tr>
<td>Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.</td>
<td>1 bottle</td>
<td>2 bottles</td>
<td>5 bottles</td>
</tr>
<tr>
<td>Incubation Buffer. Contains 0.1% sodium azide; 12 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>5 bottles</td>
</tr>
<tr>
<td>Ms IL-2 High and Low Control, recombinant Ms IL-2, lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.</td>
<td>2 vials</td>
<td>2 vials</td>
<td>4 vials</td>
</tr>
<tr>
<td>Antibody Coated Wells. 12 x 8 Well Strips.</td>
<td>1 plate</td>
<td>2 plates</td>
<td>5 plates</td>
</tr>
<tr>
<td>Ms IL-2 Biotin Conjugate (Biotin-labeled anti-IL-2). Contains 0.1% sodium azide; 6 mL per bottle.</td>
<td>1 bottle</td>
<td>2 bottles</td>
<td>5 bottles</td>
</tr>
<tr>
<td>Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.</td>
<td>1 vial</td>
<td>2 vials</td>
<td>5 vials</td>
</tr>
<tr>
<td>Streptavidin-HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (25X). 100 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>2 bottles</td>
</tr>
<tr>
<td>Stabilized Chromogen, Tetramethylbenzidine (TMB). 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Stop Solution. 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Plate Covers, adhesive strips.</td>
<td>3</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

Disposal Note
This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

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.

Avoid any skin contact with Concentrated Chromogen (TMB), Substrate Buffer, and Chromogenic Solution (TMB) and Stop Solution. In case of contact wash thoroughly with water.
## Introduction

**Purpose**
The Invitrogen Mouse Interleukin-2 (Ms IL-2) ELISA is to be used for the quantitative determination of Ms IL-2 in mouse serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms IL-2.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

**Principle of the Method**
The Invitrogen Ms IL-2 kit is a solid phase sandwich Enzyme Linked-Immunosorbent Assay (ELISA). A monoclonal antibody specific for Ms IL-2 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms IL-2 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-2 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 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-2 present in the original specimen.

**Background Information**
Interleukin-2 (IL-2), also known as T Cell Growth Factor, is a 15.5 kDa glycoprotein secreted primarily by activated T lymphocytes. The protein consists of 133 amino acids and is the product of several post translational processing steps. Transcription and release of synthesized IL-2 is stimulated by antigen or mitogen activation of mature T lymphocytes. IL-2 binds to cell surface receptors and promotes the clonal expansion of antigen-specific effector T cells. IL-2 is a T cell differentiation factor, able to induce the production of other lymphokines, such as IL-4 and interferon-γ. IL-2 promotes the growth of B cells and induction of immunoglobulin secretion. IL-2 stimulates J chain synthesis which leads to assembly and secretion of IgM. Myeloid cell populations such as macrophage precursors and primary peripheral blood monocytes express IL-2 receptors. The binding of IL-2 will cause, in these cells, proliferation, differentiation and enhancement of cytolytic activity (1, 2).
**Methods**

**Materials Needed**
- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

**But Not Provided**
- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

**Procedural Notes**
1. When not in use, kit components should be refrigerated.
2. All reagents and samples should be warmed to room temperature before use.
3. **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.
4. Samples should be collected in pyrogen/endotoxin-free tubes.
5. 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.
6. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
7. It is recommended that all standards, controls and samples be run in duplicate.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. **Do not mix or interchange different reagent lots from various kit lots.**
10. Do not use reagents after the kit expiration date.
11. 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.
12. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. 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.
14. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

**Directions for Washing**
- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.
- 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.
Preparation of Samples and Reagents

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.

Controls

Reconstitute the lyophilized Controls to the volume specified on the vial label with distilled water. Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion. See vial label for exact concentration.

Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.

Dilution of Standard

One microgram of Invitrogen recombinant mouse IL-2 equals 330,000 arbitrary units of WHO reference preparation 93/566 (NIBSC, Hertfordshire, UK, EN6 3QG).

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 10,000 pg/mL with Standard Diluent Buffer. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.050 mL of the reconstituted standard to a tube containing 0.450 mL Standard Diluent Buffer. Label as 1000 pg/mL Ms IL-2. Mix.
3. Add 0.300 mL of Standard Diluent Buffer to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL Ms IL-2.
4. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Note

Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.
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) 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.
2. Return the unused Streptavidin-HRP (100X) to the refrigerator.

<table>
<thead>
<tr>
<th># of 8-Well Strips</th>
<th>Volume of Streptavidin-HRP (100X)</th>
<th>Volume of Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20 µL solution</td>
<td>2 mL</td>
</tr>
<tr>
<td>4</td>
<td>40 µL solution</td>
<td>4 mL</td>
</tr>
<tr>
<td>6</td>
<td>60 µL solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>8</td>
<td>80 µL solution</td>
<td>8 mL</td>
</tr>
<tr>
<td>10</td>
<td>100 µL solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>12</td>
<td>120 µL solution</td>
<td>12 mL</td>
</tr>
</tbody>
</table>
Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay. 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 *Incubation Buffer* followed by 50 μL of sample (standard, serum, plasma, tissue culture supernatant or control). Add 50 μL of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty. Tap on the side of the plate to mix.
3. Pipette 50 μL of biotinylated *Ms IL-2 Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
4. Cover wells with plate cover and incubate for 2 hours at 37°C.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. (See *Directions For Washing*).
6. Add 100 μL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See *Preparation of Reagents*.
7. Cover plate with the *plate cover* and incubate for 30 minutes at room temperature.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See *Directions for Washing*.
9. Add 100 μL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
10. 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 exceeds the limits of the instrument.
11. 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.
12. 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 30 minutes after adding the *Stop Solution*.
13. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
14. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)
The following data were obtained for the various standards over the range of 0 to 1000 pg/mL Ms IL-2.

<table>
<thead>
<tr>
<th>Standard Ms IL-2 (pg/mL)</th>
<th>Optical Density (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>3.002</td>
</tr>
<tr>
<td>500</td>
<td>2.201</td>
</tr>
<tr>
<td>250</td>
<td>1.231</td>
</tr>
<tr>
<td>125</td>
<td>0.645</td>
</tr>
<tr>
<td>62.5</td>
<td>0.347</td>
</tr>
<tr>
<td>31.2</td>
<td>0.199</td>
</tr>
<tr>
<td>15.6</td>
<td>0.109</td>
</tr>
<tr>
<td>0</td>
<td>0.022</td>
</tr>
</tbody>
</table>
Performance Characteristics

Analytical Sensitivity

The minimum detectable dose of <4 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Precision

1. Intra-Assay Precision

Samples of known Ms IL-2 concentration were assayed in replicates of 32 to determine precision within an assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>114.4</td>
<td>5.5</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>391.7</td>
<td>10.2</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>771.9</td>
<td>32.8</td>
<td>4.3</td>
</tr>
</tbody>
</table>

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118.5</td>
<td>6.7</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>365.4</td>
<td>16.3</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>749.7</td>
<td>41.7</td>
<td>5.5</td>
</tr>
</tbody>
</table>

SD = Standard Deviation
CV = Coefficient of Variation

Linearity of Dilution

Mouse serum and tissue culture medium containing 1% fetal bovine serum were spiked with Ms IL-2 and serially diluted in Standard Diluent Buffer 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.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Serum</th>
<th>Measured (pg/mL)</th>
<th>Expected (pg/mL)</th>
<th>% Expected</th>
<th>Cell Culture</th>
<th>Measured (pg/mL)</th>
<th>Expected (pg/mL)</th>
<th>% Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>neat</td>
<td>Serum</td>
<td>839.0</td>
<td>839.0</td>
<td>---</td>
<td>Cell Culture</td>
<td>831.8</td>
<td>831.8</td>
<td>---</td>
</tr>
<tr>
<td>1/2</td>
<td>481.8</td>
<td>419.5</td>
<td>114.9%</td>
<td>345.2</td>
<td>415.9</td>
<td>83.0%</td>
<td>83.0%</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>246.2</td>
<td>209.8</td>
<td>117.4%</td>
<td>178.6</td>
<td>208.0</td>
<td>85.9%</td>
<td>85.9%</td>
<td></td>
</tr>
<tr>
<td>1/8</td>
<td>112.1</td>
<td>104.9</td>
<td>106.9%</td>
<td>107.9</td>
<td>104.0</td>
<td>103.7%</td>
<td>103.7%</td>
<td></td>
</tr>
<tr>
<td>1/16</td>
<td>50.1</td>
<td>52.4</td>
<td>95.5%</td>
<td>44.8</td>
<td>52.0</td>
<td>86.1%</td>
<td>86.1%</td>
<td></td>
</tr>
<tr>
<td>1/32</td>
<td>25.4</td>
<td>26.2</td>
<td>93.3%</td>
<td>23.7</td>
<td>26.0</td>
<td>91.2%</td>
<td>91.2%</td>
<td></td>
</tr>
<tr>
<td>1/64</td>
<td>13.3</td>
<td>13.1</td>
<td>101.7%</td>
<td>12.0</td>
<td>13.0</td>
<td>92.2%</td>
<td>92.2%</td>
<td></td>
</tr>
<tr>
<td>1/132</td>
<td>5.7</td>
<td>6.6</td>
<td>86.3%</td>
<td>6.1</td>
<td>6.5</td>
<td>94.6%</td>
<td>94.6%</td>
<td></td>
</tr>
</tbody>
</table>
Recovery

The recovery of Ms IL-2 added to normal mouse serum, plasma, and tissue culture medium is as follows.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>100%</td>
</tr>
<tr>
<td>Citrate Plasma</td>
<td>100%</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>105%</td>
</tr>
<tr>
<td>Heparin Plasma</td>
<td>105%</td>
</tr>
<tr>
<td>RPMI + 1% FBS</td>
<td>106%</td>
</tr>
<tr>
<td>RPMI + 10% FBS</td>
<td>105%</td>
</tr>
</tbody>
</table>

Parallelism

Natural Ms IL-2 was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein was demonstrated by the figure below and indicated that the standard accurately reflects natural Ms IL-2 content in samples.

Mouse IL-2 Parallelism

![Mouse IL-2 Parallelism Graph](image)

Antigenic Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Invitrogen Ms IL-2 kit. The following substances were tested and found to have no cross-reactivity: human IL-2, IL-5, IL-12, GM-CSF, RANTES; mouse IL-1β, IL-3, IL-4, IL-6, IL-10, IFN-γ, MCP-1, TNF-α; rat IL-1α, IL-1β, IL-2, IL-4, IL-10, IFN-γ, TNF-α.
Sixteen sera, sixteen plasma (heparin) samples were evaluated in this assay. All samples measured <15.6 pg/mL (the lowest Ms IL-2 standard).

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for Ms IL-2 released.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-A (5 ( \mu )g/mL) 6 hr</td>
<td>605 pg/mL</td>
</tr>
<tr>
<td>PMA (50 ng/mL), Ionophore (250 ng/mL) 6 hr</td>
<td>555 pg/mL</td>
</tr>
<tr>
<td>PMA (50 ng/mL), Ionophore (250 ng/mL) 24 hr</td>
<td>2724 pg/mL</td>
</tr>
<tr>
<td>LPS (1 ( \mu )g/mL) 24 hr</td>
<td>827 pg/mL</td>
</tr>
</tbody>
</table>

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-2 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

**Cause:** Insufficient washing and/or draining of wells after washing. Solution containing either biotin or HRP-Conjugate can elevate the background if residual is left in the well.

**Solution:** Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.

**Cause:** Contamination of substrate solution with metal ions or oxidizing reagents.

**Solution:** Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

**Cause:** Contamination of pipette, dispensing reservoir or substrate solution with HRP Conjugate.

**Solution:** Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

**Cause:** Incubation time is too long or incubation temperature is too high.

**Solution:** Reduce incubation time and/or temperature.

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Elevated sample/standard ODs

**Cause:** Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.

**Solution:** Follow the protocol instructions regarding the dilution of the standard.

**Cause:** Incorrect dilution of the HRP Conjugate.

**Solution:** Warm solution of HRP Conjugate concentrate to room temperature, draw up slowly and wipe tip with laboratory wipe to remove excess. Dilute ONLY in SAV diluent provided.

**Cause:** Incubation times extended.

**Solution:** Follow incubation times outlined in protocol.

**Cause:** Incubations carried out at 37°C when RT is dictated.

**Solution:** Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

---

Poor standard curve

**Cause:** Improper preparation of standard stock solution.

**Solution:** 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.

**Cause:** Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted.

**Solution:** NEVER substitute any components from another kit.

**Cause:** Errors in pipetting the standard or subsequent steps.

**Solution:** 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 develops

Cause: Reagents not at RT (25 ± 2°C) at start of assay.
Solution: Allow ALL reagents to warm to RT prior to commencing assay.

Cause: Incorrect storage of components, e.g., not stored at 2 to 8°C.
Solution: Store all components exactly as directed in protocol and on labels.

Cause: Working HRP Conjugate solution made up longer than 15 minutes before use in assay.
Solution: Use the diluted HRP Conjugate within 15 minutes of dilution.

Cause: TMB solution lost activity.
Solution 1: 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.
Solution 2: Avoid contact of the TMB solution with items containing metal ions.

Cause: Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.
Solution: Please contact Technical Support for advice when using alternative sample types.

Cause: Wells have been scratched with pipette tip or washing tips.
Solution: Use caution when dispensing and aspirating into and out of microwells.

Poor Precision

Cause: Errors in pipetting the standards, samples or subsequent steps.
Solution: 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.

Cause: Repetitive use of tips for several samples or different reagents.
Solution: Use fresh tips for each sample or reagent transfer.

Cause: Wells have been scratched with pipette tip or washing tips.
Solution: 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 www.invitrogen.com/ELISA.

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References

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### Explanation of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue Number</td>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>RUO</td>
<td>Research Use Only</td>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td></td>
<td>Use by</td>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
<td>EC REP</td>
<td>European Community authorised representative</td>
</tr>
<tr>
<td>[-]</td>
<td>Without, does not contain</td>
<td>[+]</td>
<td>With, contains</td>
</tr>
<tr>
<td><img src="image" alt="Protect from Light" /></td>
<td>Protect from light</td>
<td><img src="image" alt="Consult accompanying documents" /></td>
<td>Consult accompanying documents</td>
</tr>
<tr>
<td><img src="image" alt="Direct the user to consult instructions for use (IFU), accompanying the product." /></td>
<td>Directs the user to consult instructions for use (IFU), accompanying the product.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This kit has been improved. The following are the changes between the old kit and this kit. The assay procedure has not changed with the exception of the use of incubation buffer for all samples. See below.

<table>
<thead>
<tr>
<th>Changes</th>
<th>Old Kit</th>
<th>New Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery – Serum</td>
<td>89%</td>
<td>100%</td>
</tr>
<tr>
<td>Recovery – Citrate Plasma</td>
<td>No claim</td>
<td>100%</td>
</tr>
<tr>
<td>Recovery – EDTA Plasma</td>
<td>No claim</td>
<td>105%</td>
</tr>
<tr>
<td>Recovery – Heparin Plasma</td>
<td>89%</td>
<td>105%</td>
</tr>
<tr>
<td>Recovery – RPMI + 1% FBS</td>
<td>93%</td>
<td>106%</td>
</tr>
<tr>
<td>Recovery – RPMI + 10% FBS</td>
<td>99%</td>
<td>105%</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>&lt; 8 pg/mL</td>
<td>&lt; 4 pg/mL</td>
</tr>
<tr>
<td>Assay procedure - Incubation Buffer</td>
<td>Only serum and plasma samples used incubation buffer.</td>
<td>All samples use incubation buffer.</td>
</tr>
</tbody>
</table>
Mouse IL-2 Assay Summary

Add 50 μL Incubation Buffer
Add 50 μL of standards, controls and samples

Add 50 μL of Biotin Conjugate
Incubate for 2 hr at 37°C

↓
aspirate and wash 4x

Incubate 100 μL of Streptavidin-HRP Working Solution for 30 min at RT

↓
aspirate and wash 4x

Incubate 100 μL of Stabilized Chromogen for 30 min at RT

↓

Add 100 μL of Stop Solution
Read at 450 nm