PRINCIPLE OF THE ASSAY
This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IL-2 has been pre-coated onto a microplate. Standards, Controls, and samples are pipetted into the wells and any mouse IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse IL-2 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE
- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION
The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

TECHNICAL HINTS
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.

REAGENTS
Mouse IL-2 Microplates (Part 890327) - Two 96 well polystyrene microplates (12 strips of 8 wells) coated with polyclonal antibody specific for mouse IL-2.
Mouse IL-2 Conjugate Concentrate (Part 890328) - 1 mL of a 23-fold-concentrated solution containing polyclonal antibody against mouse IL-2 conjugated to horseradish peroxidase, with preservatives.
Type 2 Conjugate Diluent (Part 895463) - 23 mL of diluent for diluting the conjugate concentrate, with preservatives.
Mouse IL-2 Standard (Part 890329) - 3 vials (2.0 ng/vial) of recombinant mouse IL-2 in a buffered protein base, with preservatives, lyophilized.
Mouse IL-2 Control (Part 890451) - 3 vials of recombinant mouse IL-2 in a buffered protein base with preservatives, lyophilized. The concentration range of mouse IL-2 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the Control label.
Assay Diluent RD1-14 (Part 895180) - 12.5 mL of a buffered protein solution, with preservatives. Contains a precipitate. Mix well before use.
Calibrator Diluent RDST (Part 895175) - 21 mL of a buffered protein solution, with preservatives.
Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of a buffered surfactant, with preservatives.
Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.
Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).
Stop Solution (Part 895174) - 23 mL of a diluted hydrochloric acid solution.
Plate Covers (Part 640197) - 8 adhesive plate sealers.
**STORAGE**

<table>
<thead>
<tr>
<th>Unopened Kit</th>
<th>Store at 2 - 8° C. Do not use beyond kit expiration date.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted mouse IL-2 Conjugate</td>
<td>May be stored for up to 1 week at 2 - 8° C.*</td>
</tr>
<tr>
<td>Diluted Wash Buffer</td>
<td></td>
</tr>
<tr>
<td>Stop Solution</td>
<td></td>
</tr>
<tr>
<td>Calibrator Diluent RDST</td>
<td></td>
</tr>
<tr>
<td>Assay Diluent RD1-14</td>
<td>May be stored for up to 1 month at 2 - 8° C.*</td>
</tr>
<tr>
<td>Conjugate Concentrate</td>
<td></td>
</tr>
<tr>
<td>Conjugate Diluent</td>
<td></td>
</tr>
<tr>
<td>Unmixed Color Reagent A</td>
<td>Use a new Standard and Control for each assay.</td>
</tr>
<tr>
<td>Unmixed Color Reagent B</td>
<td></td>
</tr>
<tr>
<td>mL L-2 Standard (1000 pg/mL)</td>
<td></td>
</tr>
<tr>
<td>mouse IL-2 Control</td>
<td></td>
</tr>
<tr>
<td>Microplate Wells</td>
<td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td>
</tr>
</tbody>
</table>

*Provided this is within the expiration date of the kit.

**OTHER SUPPLIES REQUIRED**
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- 1000 mL graduated cylinder.
- Deionized or distilled water.
- Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer.
- Polypropylene tubes.

**SAMPLE COLLECTION AND STORAGE**

**Cell Culture Supernatants** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

**Serum** - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at approximately 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

**Note:** Grossly hemolyzed or lipemic samples may not be suitable for measurement of mouse IL-2 with this assay.

**REAGENT PREPARATION**

Bring all reagents to room temperature before use.

**Mouse IL-2 Kit Control** - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

**Mouse IL-2 Conjugate** - To prepare enough conjugate for one plate, add 0.5 mL Conjugate Concentrate to 11.0 mL Calibrator Diluent. Use a sterile container and protect from light.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough wash buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

**Mouse IL-2 Standard** - Reconstitute the Mouse IL-2 Standard with 2.0 mL of Calibrator Diluent RDST. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

**Use polypropylene tubes.** Pipette 200 µL of Calibrator Diluent RDST into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-2 Standard serves as the high standard (1000 pg/mL). Calibrator Diluent RDST serves as the zero standard (0 pg/mL).

![Diagram](image-url)
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards and controls be assayed in duplicate.

1. Prepare reagents, standards and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.

3. Add 50 μL of Assay Diluent RD1-14 to each well. RD1-14 may contain undissolved material even when mixed well before its use.

4. Add 50 μL of Standard, Control or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. Plate layouts are provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 μL of Mouse IL-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.

8. Add 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.

9. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

PROCEDURE SUMMARY AND CHECKLIST

1. ☐ Bring all reagents to room temperature.
☐ Prepare reagents, standards and samples as instructed.
☐ Return unused components to storage temperature as indicated in the instructions.

2. ☐ Add 50 μL Assay Diluent to the center of each well.

3. ☐ Add 50 μL Standard, Control, or sample to the center of each well.
☐ Tap plate gently for one minute.
☐ Cover the plate and incubate 2 hours at room temperature.

4. ☐ Aspirate and wash each well five times.

5. ☐ Add 100 μL Conjugate to each well.
☐ Cover the plate and incubate 2 hours at room temperature.

6. ☐ Aspirate and wash each well five times.

7. ☐ Add 100 μL Substrate Solution to each well. Incubate 30 minutes at room temperature. Protect from light.

8. ☐ Add 100 μL Stop Solution to each well.

9. ☐ Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).
CALCULATION OF RESULTS
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a log-log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

![](chart.png)

<table>
<thead>
<tr>
<th>(pg/mL)</th>
<th>O.D.</th>
<th>Average</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.050</td>
<td>0.050</td>
<td>—</td>
</tr>
<tr>
<td>0.03</td>
<td>0.068</td>
<td>0.068</td>
<td>0.033</td>
</tr>
<tr>
<td>0.17</td>
<td>0.117</td>
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<tr>
<td>0.25</td>
<td>0.182</td>
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<td>0.132</td>
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<tr>
<td>0.35</td>
<td>0.320</td>
<td>0.320</td>
<td>0.270</td>
</tr>
<tr>
<td>0.55</td>
<td>0.593</td>
<td>0.593</td>
<td>0.543</td>
</tr>
<tr>
<td>1.05</td>
<td>1.060</td>
<td>1.060</td>
<td>1.010</td>
</tr>
<tr>
<td>1.852</td>
<td>1.838</td>
<td>1.865</td>
<td>1.815</td>
</tr>
</tbody>
</table>

PRECISION
Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty assays to assess inter-assay precision.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (pg/mL)</td>
<td>70.4</td>
<td>187</td>
<td>595</td>
<td>70.9</td>
<td>175</td>
<td>559</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3.5</td>
<td>10.0</td>
<td>23.4</td>
<td>3.6</td>
<td>9.6</td>
<td>24.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.5</td>
<td>5.3</td>
<td>3.9</td>
<td>5.1</td>
<td>5.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

RECOVERY
The recovery of mouse IL-2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture supernate (n=6)</td>
<td>102</td>
<td>84 - 110%</td>
</tr>
<tr>
<td>Mouse serum (n=6)</td>
<td>102</td>
<td>96 - 108%</td>
</tr>
</tbody>
</table>