CALCULATION OF RESULTS
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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. The data may be linearized by plotting the log of the mouse IL-2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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

SPECIFICITY
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

<table>
<thead>
<tr>
<th>Recombinant mouse:</th>
<th>Recombinant human:</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>IL-13</td>
</tr>
<tr>
<td>G-CSF</td>
<td>IL-2</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>IL-2 Rα</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>IL-2 Rβ</td>
</tr>
<tr>
<td>IL-1α</td>
<td>M-CSF</td>
</tr>
<tr>
<td>IL-1β</td>
<td>MIP-1α</td>
</tr>
<tr>
<td>IL-3</td>
<td>MIP-1β</td>
</tr>
<tr>
<td>IL-4</td>
<td>MIP-2</td>
</tr>
<tr>
<td>IL-5</td>
<td>SCF</td>
</tr>
<tr>
<td>IL-6</td>
<td>TNF-α</td>
</tr>
<tr>
<td>IL-7</td>
<td>Tpo</td>
</tr>
<tr>
<td>IL-9</td>
<td>VEGF</td>
</tr>
</tbody>
</table>

A sample containing 50 ng/mL of recombinant rat IL-2 read as 250 pg/mL (0.5% cross-reactivity).

TECHNICAL HINTS & LIMITATIONS
- We recommend the use of R&D Systems’ Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare the Block Buffer for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent and Block Buffer is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.
- It is important that the Reagent Diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING
Note: For more detailed troubleshooting, please visit: www.RnDSystems.com/ELISADevelopment

Poor Standard Curve
- Impure BSA used for Reagent Diluent preparation and Block Buffer.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

Poor Precision
- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Low or No Color Development
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation and Block Buffer.

MANUFACTURED AND DISTRIBUTED BY:
USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

www.RnDSystems.com
DuoSet Ancillary Reagent Kit 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate
sealers, substrate solution, stop solution, plate coating buffer (PBS),
wash buffer, and Reagent Diluent Concentrate 2.

The components listed above may be purchased separately:
96 well microplates: (R&D Systems, Catalog # DY990).
Plate Sealers: (R&D Systems, Catalog # DY992).
PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4,
pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY006).
Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).
Block Buffer: 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY995).
Wear protective gloves, clothing, eye, and face protection. Wash
eye, and respiratory irritation. Avoid breathing fumes.
The Color Reagent B suggested for use with this kit may cause skin,
as well as respiratory allergy. Avoid breathing mist.
Some components in this kit contain ProClin® which may cause an
allergic skin reaction. Avoid breathing mist.
The Stop Solution suggested for use with this kit is an acid solution.
The Color Reagent B suggested for use with this kit may cause skin,
which can irritate eyes and respiratory system. Avoid breathing fumes.
Wear protective gloves, clothing, eye, and face protection. Wash
hands thoroughly after handling. Please refer to the MSDS on our
website prior to use.

OTHER MATERIALS & SOLUTIONS REQUIRED

The Color Reagent A (H2O2) and Color
Quality of BSA is critical (see Technical Hints).

Reagent Diluent:
(20 mM Trizma base, 150 mM NaCl) pH 7.2-7.4, 0.2 μm filtered.
0.1% BSA, 0.05% Tween 20 in Tris-buffered Saline
Reagent Diluent:
(R&D Systems, Catalog # DY995).

Wash Buffer:
0.05% Tween® 20 in PBS, pH 7.2-7.4
(R&D Systems, Catalog # DY006).
Wear protective gloves, clothing, eye, and face protection. Wash
eye, and respiratory irritation. Avoid breathing fumes.
The Stop Solution suggested for use with this kit is an acid solution.
The Color Reagent B suggested for use with this kit may cause skin,
as well as respiratory allergy. Avoid breathing mist.
Some components in this kit contain ProClin® which may cause an
allergic skin reaction. Avoid breathing mist.

Plate Preparation
1. Dilute the Capture Antibody to the working concentration
in PBS without carrier protein. Immediately coat a 96-well
microplate with 100 μL per well of the diluted Capture
Antibody. Seal the plate and incubate overnight at room
temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the
process two times for a total of three washes. Wash by filling
each well with Wash Buffer (400 μL) using a squirt bottle,
manifold dispenser, or autosampler. Complete removal of liquid
at each step is essential for good performance. After the last
wash, remove any remaining Wash Buffer by aspirating or by
inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well.
Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now
ready for sample addition.

Calibration
This DuoSet is calibrated against a highly purified E. coli-expressed
recombinant mouse IL-2 produced at R&D Systems.

Materials Provided & Storage Conditions

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>PART #</th>
<th>CATALOG #</th>
<th>CATALOG #</th>
<th>STORAGE OF OPENED/ RECONSTITUTED MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse IL-2 Capture Antibody</td>
<td>840137</td>
<td>DY402-05</td>
<td>DY402</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
<tr>
<td>Mouse IL-2 Detection Antibody</td>
<td>840138</td>
<td>1 vial</td>
<td>3 vials</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
<tr>
<td>Mouse IL-2 Standard</td>
<td>840139</td>
<td>1 vial</td>
<td>3 vials</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
<td>893975</td>
<td>1 vial</td>
<td>3 vials</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
</tbody>
</table>

Calibration

The Color Reagent B suggested for use with this kit may cause skin,
as well as respiratory allergy. Avoid breathing mist.
Some components in this kit contain ProClin® which may cause an
allergic skin reaction. Avoid breathing mist.

General ELISA Protocol

Wash Buffer:
0.05% Tween® 20 in PBS, pH 7.2-7.4
(R&D Systems, Catalog # WA126).

Substrate Solution:
1:1 mixture of Color Reagent A (H2O2) and Color
Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).
Plate Sealers:
(20 mM Trizma base, 150 mM NaCl) pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY006).
96 well microplates:
(R&D Systems, Catalog # DY990).

Catalog # 840137
8000 pg/mL
5000 pg/mL
2500 pg/mL
1250 pg/mL
625 pg/mL
313 pg/mL
156 pg/mL

Catalog # 840138
500 μL
500 μL
500 μL
500 μL
500 μL
500 μL
500 μL

Catalog # 840139
1000 μL
1000 μL
1000 μL
500 μL
500 μL
500 μL
500 μL

Catalog # 893975
1 vial
1 vial
1 vial
1 vial
1 vial
1 vial
1 vial

Catalog # 840137
1 vial
1 vial
1 vial
1 vial
1 vial
1 vial
1 vial

Catalog # 840138
3 vials
3 vials
3 vials
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3 vials
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Catalog # 840139
3 vials
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Catalog # 893975
3 vials
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3 vials
3 vials

This DuoSet Ancillary Reagent Kit 2 is calibrated against a highly purified E. coli-expressed recombinant mouse IL-2 produced at R&D Systems.

Wear protective gloves, clothing, eye, and face protection. Wash
eye, and respiratory irritation. Avoid breathing fumes.
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1. Dilute the Capture Antibody to the working concentration
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Antibody. Seal the plate and incubate overnight at room
temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the
process two times for a total of three washes. Wash by filling
each well with Wash Buffer (400 μL) using a squirt bottle,
manifold dispenser, or autosampler. Complete removal of liquid
at each step is essential for good performance. After the last
wash, remove any remaining Wash Buffer by aspirating or by
inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well.
Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now
ready for sample addition.

Assay Procedure
1. Add 100 μL of sample or standards in Reagent Diluent, or an
appropriate diluent, per well. Cover with an adhesive strip and
incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 μL of the Detection Antibody, diluted in Reagent
Diluent, to each well. Cover with a new adhesive strip and
incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 μL of the working dilution of Streptavidin-HRP to each
well. Cover the plate and incubate for 20 minutes at room
temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for
20 minutes at room temperature. Avoid placing the plate in
direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate
to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

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