**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 TRANCE 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.

**Recombinant human:**
CD40 Ligand
TRANCE

Recombinant mouse RANK does not cross-react in this assay but does interfere at concentrations > 1.56 ng/mL.

Recombinant mouse OPG does not cross-react in this assay but does interfere at concentrations > 250 pg/mL.

**TECHNICAL HINTS & LIMITATIONS**

- We recommend the use of R&D Systems’® Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent 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 A.
- 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.
- 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.
- 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.

**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.

**TRoubleSHOOTING**

- Incorrect incubation times or temperatures.
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

**TROUBLESHOOTING**

- Incorrect incubation times or temperatures.
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.

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www.RnDSystems.com
OTHER MATERIALS & SOLUTIONS REQUIRED

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).
- Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered (R&D Systems®, Catalog # DY999).
- Substrate Solution: 1:1 mixture of Color Reagent A (H2O2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems®, Catalog # DY994).
- Stop Solution: 2 N H2SO4 (R&D Systems®, Catalog # DY990).

PRECAUTIONS

Some components in this kit contain a preservative 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, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

CALIBRATION

This DuoSet® is calibrated against a highly purified NS0-expressed recombinant mouse TRANCE 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># VIALS</th>
<th>STORAGE OF OPENED/RECONSTITUTED MATERIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse TRANCE Capture Antibody</td>
<td>840818</td>
<td>1 vial</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
<tr>
<td>Mouse TRANCE Detection Antibody</td>
<td>840819</td>
<td>1 vial</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
<tr>
<td>Mouse TRANCE Standard</td>
<td>840820</td>
<td>3 vials</td>
<td></td>
</tr>
<tr>
<td>Streptavidin-HRP A</td>
<td>895003</td>
<td>1 vial</td>
<td>Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.</td>
</tr>
</tbody>
</table>

REAGENT PREPARATION

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP A: 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Goat Anti-Mouse TRANCE Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Mouse TRANCE Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Mouse TRANCE Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 μL of high standard per plate assayed at the concentration indicated on the C of A.

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