**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 human TGF-β3 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. Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**NOTES**

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 human TGF-β3 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. Since 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 human:</th>
<th>Recombinant amphibian:</th>
<th>Natural:</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>TGF-β5</td>
<td>porcine TGF-β1</td>
</tr>
<tr>
<td>Latent TGF-β1</td>
<td>TGF-β5</td>
<td>porcine TGF-β2</td>
</tr>
<tr>
<td>TGF-β1-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following factors prepared at 50 ng/mL did not cross-react in this assay but did interfere as listed below:

<table>
<thead>
<tr>
<th>Recombinant</th>
<th>Expected TGF-β3 Concentration</th>
<th>Measured TGF-β3 Concentration</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHF-Fc RePl</td>
<td>200 pg/mL</td>
<td>340 pg/mL</td>
<td>42.9</td>
</tr>
<tr>
<td>Human TGF-β</td>
<td>100 pg/mL</td>
<td>194 pg/mL</td>
<td>46.2</td>
</tr>
<tr>
<td>Human TGF-β RII</td>
<td>25 pg/mL</td>
<td>164 pg/mL</td>
<td>84.3</td>
</tr>
<tr>
<td>Mouse TGF-β RIIF</td>
<td>50 pg/mL</td>
<td>117 pg/mL</td>
<td>56.5</td>
</tr>
</tbody>
</table>

**INTENDED USE**

For the development of sandwich ELISAs to measure natural and recombinant human Transforming Growth Factor beta 3 (TGF-β3). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay.

Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least fifteen 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product. Refer to the Certificate of Analysis for component concentrations as they may vary. For research use only. Not for use in diagnostic procedures.
Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

**MATERIALS PROVIDED & STORAGE CONDITIONS**

- **Duoset® Ancillary Reagent Kit (2.5 plates):** (R&D Systems®, Catalog # DY990).
  - Contains 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), and Reagent Diluent Concentrate 2.
- **The components listed above may be purchased separately:**
  - 96 well microplates: (R&D Systems®, Catalog # DY992).
  - 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 # DY995).
  - Quality of BSA is critical (see Technical Hints).
  - Substrate Solution: 1:1 mixture of Color Reagent A (H2O) and Color Reagent B (Tetramethylbenzidine) (R&D Systems®, Catalog # DY999).
- **Stop Solution:** 2 N H2SO4, (R&D Systems®, Catalog # DI994).
- Also available for purchase:
  - Sample Activation Kit: 3 vials (10 mL/vial) of 1N HCl and 3 vials (10 mL/vial) of 1.2 N NaOH/0.5 M HEPES (R&D Systems®, Catalog # DYO10).

**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.

**REAGENT PREPARATION**

- **Sample Activation Kit:**
  - To activate latent TGF-β3 to the immunoreactive form, use Sample Activation Kit 1 (R&D Systems®, Catalog # DYO10) or perform its own serum diluent validation.
  - It is recommended that all standards and samples be assayed in duplicate.
  - The use of PBS from tablets may interfere in this assay.

**Calibration**

- This Duoset® is calibrated against a highly purified S/21-expressed recombinant human TGF-β3 produced at R&D Systems®.

**ACTIVATION REAGENT PREPARATION**

To activate latent TGF-β3 to the immunoreactive form, use Sample Activation Kit 1 (R&D Systems®, Catalog # DYO10) or prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month.

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**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.**
- **The Reagent Diluent used to construct the standard curve must be optimized for each sample type. The formulation given may be suitable for most cell culture supernates. Each laboratory should perform its own serum diluent validation.**
- **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 rinsed 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.**
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**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.**

- **Low or No color Development**
  - **Unequal volumes added to wells/pipetting error.**
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