GENERAL ELISA PROTOCOL

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 autowasher. 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. Avoid placing the plate in direct light.

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 B 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.

TECHNICAL HINTS & LIMITATIONS

- Incorrect incubation times or temperatures.
- Inadequate volume of substrate added to wells.
- Unequal mixing of reagents.
- 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.

SPECIFICITY

The factors below were assayed and exhibited the following cross-reactivity:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Concentration Tested (pg/mL)</th>
<th>Observed Value (pg/mL)</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTGF-β1</td>
<td>50,000</td>
<td>151</td>
<td>8.3%</td>
</tr>
<tr>
<td>pTGF-β1.1</td>
<td>250,000</td>
<td>134</td>
<td>6.7%</td>
</tr>
<tr>
<td>pTGF-β2</td>
<td>50,000</td>
<td>74</td>
<td>0.15%</td>
</tr>
<tr>
<td>pTGF-β3</td>
<td>50,000</td>
<td>161</td>
<td>0.96%</td>
</tr>
<tr>
<td>pTGF-β5</td>
<td>50,000</td>
<td>184</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

A sample containing 25 ng/mL of recombinant human latent TGF-β1 reads as 37.4 pg/mL (0.15% cross-reactivity).

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human Transforming Growth Factor beta 1 (TGF-β1). 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 five 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.

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MATERIALS PROVIDED & STORAGE CONDITIONS

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

OTHER MATERIALS & SOLUTIONS REQUIRED

DuoSet® Ancillary Reagent Kit 1 (5 plates): (R&D Systems®, Catalog # DY700) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), block buffer, wash buffer, and Reagent Diluent Concentrate 1.

The components listed above may be purchased separately:
- 96 well microplates: (R&D Systems®, Catalog # DY990).
- Plate Sealers: (R&D Systems®, Catalog # DY992).
- Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4
- Block Buffer: 5% Tween 20 in PBS, pH 7.2-7.4
- Stop Solution: 2 N H2SO4, (R&D Systems®, Catalog # DY994).
- Plate Sealers: (R&D Systems®, Catalog # DY992).

Also available for purchase:
- Sample Activation Kit 1: 3 vials (10 mL/vial) of 1N HCl and 3 vials (10 mL/vial) of 1.2 N NaOH/0.5M HEPES (R&D Systems®, Catalog # DY010).

CALIBRATION

This DuoSet® is calibrated against a highly purified CHO cell-expressed recombinant human TGF-β1 produced at R&D Systems®.

Activation Reagent Preparation

To activate latent TGF-β1 to the immunoreactive form, use Sample Activation Kit 1 (R&D Systems®, Catalog # DY010) 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.

Caution: Wear protective clothing and safety glasses during preparation or use of these reagents.

1 N HCl (100 mL) - To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCl. Mix well.
1.2 N NaOH/0.5 M HEPES (100 mL) - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water.

TGF-β1 Sample Activation

To activate latent TGF-β1 to immunoreactive TGF-β1, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.2-7.6). Use polypropylene test tubes.

Note: Do not activate the kit standards. The kit standards contain active TGF-β1.

Cell Culture Supernate Notes

Significant levels of latent TGF-β1 are found in bovine, porcine, equine, and caprine sera. The reported levels of TGF-β1 in bovine and fetal bovine sera can be as high as 16 ng/mL after activation (1, 2). Therefore, conditioned medium containing 10% fetal bovine serum can be expected to have a TGF-β1 concentration of about 1600 pg/mL. The background level of TGF-β1 in control medium can be determined and subtracted from samples of conditioned medium. As an alternative, the background level of TGF-β1 in medium can be lowered using the following procedure prior to assaying (reference 1 with modifications). After growth to confluence in medium containing 10% serum, the medium is changed to serum-free medium supplemented with 200 µg/mL crystalline BSA with four changes of medium over 12-24 hours. Cells are then switched to medium alone or medium containing 200 µg/mL crystalline BSA. Particular cell lines may require specific additions to the serum-free medium for maintenance. After 24 hours, the serum-free conditioned medium is clarified by centrifugation and samples are stored at ≤ -70 °C. Optionally, 2.0 µg/mL aprotinin, leupeptin, pepstatin A, and 120 µg/mL PMSF can be added before freezing. Thawed or fresh samples of serum-free or serum-containing conditioned medium are processed further as described above. If bovine serum added as a supplement to conditioned media exceeds 5%, further dilute the activated sample at least 2-fold using Reagent Diluent. The dilution as a result of the sample activation procedure (1:4-fold) should be taken into consideration in the final concentration of TGF-β1 in cell media samples.

Plasma Note

TGF-β1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of TGF-β1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

REFERENCES


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