Human/Mouse TGF beta 1 Uncoated ELISA

Catalog Number: 88-8350
Also known as: Transforming Growth Factor beta 1, TGF-b1

Product Information

Contents: Human/Mouse TGF beta 1 Uncoated ELISA
Catalog Number: 88-8350
Sensitivity: 8 pg/mL
Standard Curve Range: 1000-8 pg/mL

Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
Batch Code: Refer to vial
Use By: Refer to vial

Description

This Human/Mouse TGF beta 1 Uncoated ELISA set contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of human or mouse TGF beta 1 protein levels from samples including serum, plasma, and supernatants from cell cultures. This second generation kit has increased sensitivity with a range of 8-1000 pg/mL.

Transforming Growth Factor beta (TGF beta) is a pleiotropic cytokine which exists in five isoforms, known as TGF beta 1-5, with homologies of 70-80% and no homology to TGF alpha. TGF beta 1 is the most abundant isoform and is ubiquitously expressed, while other isoforms are expressed in a more restricted manner. TGF beta 1 is highly conserved, with 100% sequence homology between the human, simian, bovine, porcine, and chicken proteins and 99% homology between the human and murine proteins. It is highly expressed in platelets and also produced by macrophages, lymphocytes, endothelial cells, chondrocytes, and leukemic cells.

TGF beta 1 is synthesized as a long precursor polypeptide, which is cleaved to yield the mature protein and the Latency Associated Peptide (LAP). LAP and mature TGF beta 1 remain non-covalently associated through secretion, forming homodimers known as the Small Latent Complex (SLC). Secretion can be induced by steroids, retinoids, EGF, NGF, vitamin D3, and IL-1. The bioactivity of mature TGF beta 1 is dependent on its release from LAP by conformational changes and proteolytic processing. Its activities include inhibition of cell growth in epithelial cells, endothelial cells, fibroblasts, neurons, lymphoid cells, and other hematopoietic cell types. TGF beta 1 also inhibits the proliferation of T cells and NK cells, downregulates the activities of activated macrophages, and blocks the anti-tumor activity of IL-2-bearing lymphokine-activated killer (LAK) cells. Recently, TGF beta 1 has been found to have a critical role in the development of regulatory T cells and act as a costimulatory factor for expression of Foxp3.

Dendritic cells exposed to tumors have been reported to secrete TGF beta 1 and stimulate the differentiation of CD4+CD25+ Treg cells from peripheral CD4+CD25- progeny. TGF beta 1-induced regulatory T cells have been termed iTreg.
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Components
Capture Antibody: Pre-titrated, purified antibody
Detection Antibody: Pre-titrated, biotin-conjugated antibody
Standard: Recombinant cytokine for generating standard curve and calibrating samples
Coating Buffer: 10X PBS ELISA Coating Buffer
Assay Diluent: 5X Concentrated
Detection Enzyme: Pre-titrated Avidin-HRP
Substrate Solution: Tetramethylbenzidine (TMB) Solution
96-well Plates: Corning Costar flat-bottom plates (included with Cat. #s ending in -22, -76, and -86)

Applications Reported
This ELISA set is for the quantitative detection of human and mouse TGF beta 1 in serum, plasma, and tissue culture supernatant samples.

Applications Tested
The Human/Mouse TGF beta 1 Uncoated ELISA (2nd Generation) recognizes the mature/active form of TGF beta 1 without association with Latency Associated Peptide (LAP). Most samples will require acid-treatment and neutralization to remove LAP from TGF beta 1 prior to evaluation in this assay. Samples should be tested in the assay immediately after acid treatment and neutralization. It is also possible that some serum and plasma samples may contain low levels of immunoreactive TGF beta 1 that has disassociated from LAP. Naturally occurring, free TGF beta 1 may be measurable in this assay by evaluating samples without acid treatment. See the "Experimental Procedure" section of the protocol.

The 5X ELISA Diluent provided in this assay contains 10% Fetal Bovine Serum (FBS) when diluted to its working concentration. FBS is a natural source of bovine TGF beta 1, which is detectable in this ELISA. It is recommended that a sample of prepared 1X ELISA Diluent be acidified and neutralized, as described in the "Experimental Procedure" section of the protocol, then run in the ELISA to quantify basal levels of bovine TGF beta 1 present in the diluent. This value can be subtracted from any samples diluted in this buffer after analysis. When testing tissue culture supernatants, it is also recommended that the user run a similar control if the cells will be cultured in medium prepared with FBS. Please use 1X ELISA Diluent that has not been treated with acid for diluting any samples for running in this ELISA.

This assay was validated for the detection of endogenous human TGF beta 1 using supernatant collected from a culture of normal peripheral blood monocytes stimulated using Cell stimulation Cocktail (500X) (cat. 00-4970) that contains PMA and ionomycin. Detection of endogenous mouse protein was tested using supernatant from a culture of splenocytes stimulated in the same manner. Due to high circulating levels of TGF beta 1 present in normal donors, it is recommended that acid-treated serum and plasma samples be diluted at least 5-fold prior to evaluation in this assay. This dilution is not required if measurement of naturally occurring free TGF beta 1 is desired, as those levels will be much lower.

This assay was evaluated for specificity on a panel of 72 recombinant cytokines at 100 ng/mL. At this concentration, 0.1% cross-reactivity to human TGF beta 2 was observed, with none to TGF beta 3 or any other cytokines on the panel.

References
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Related Products
00-4970 Cell Stimulation Cocktail (500X)
00-0400 ELISA Wash Buffer - 10 x 1L Packets
00-4201 1X TMB Solution
00-4202 5X ELISA/ELISPOT Diluent
Enzyme Linked Immunosorbent Assay (ELISA)

Protocol: Uncoated ELISA
The following protocol is a general guideline for the TGF beta 1 Uncoated Sets

Materials Provided
- Please refer to the Certificate of Analysis (C of A) for components

Other Materials Needed
- Solutions for activating samples (not needed for standards)
  - 1 N HCl
  - 1 N NaOH
- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or Thermo Fisher ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M H3PO4 (recommended) or 2N H2SO4
- Pipettes
- Refrigerator
- Frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp™)

NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp (Cat. No. 44-2404) 96-well plates

- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

NOTE: To ensure optimal results from this Uncoated ELISA Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Time Requirements
- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

Experimental Procedure
1. Coat Corning Costar 9018 (or Nunc Maxisorp™) ELISA plate with 100 µL/well of capture antibody in 1X Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 2-8°C.
2. Aspirate wells and wash 3 times with >250 µL/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X ELISA/ELISPOT Diluent with 4 parts DI water.* Block wells with 200 µL/well of 1X ELISA/ELISPOT Diluent. Incubate at room temperature for 1 hour.
4. Optional: Aspirate and wash at least once with Wash Buffer.
5. Acid Activation of Samples: To activate latent TGFß1 to the immunoreactive form, the samples (but not standards) must be acidified, and then neutralized. Animal serum used in culture media may contain high levels of latent TGFß1, so controls should be run to determine baseline concentrations of TGFß1 in culture media.
   - Tissue culture supernatants: Per 100 ul of sample, add 20 ul of 1N HCl; incubate 10 minutes at room temperature, then neutralize with 20 ul of 1N NaOH. [When calculating final sample concentration, correct to the dilution factor of 1.4.]
   - Serum or plasma: Dilute 1:5 in FBS*, then treat as above for supernatants
6. Using 1X ELISA/ELISPOT Diluent, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µL/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µL/well of your samples to the appropriate wells, diluting them at least 2-fold in 1X ELISA/ELISPOT Diluent *. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 2-8°C for maximal sensitivity).
7. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
8. Add 100 µL/well of detection antibody diluted in 1X ELISA/ELISPOT Diluent * (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.

9. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.

10. Add 100 µL/well of Avidin-HRP* diluted in 1X ELISA/ELISPOT Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.

11. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes**.

12. Add 100 µL/well of 1X TMB Solution to each well. Incubate plate at room temperature for 15 minutes.

13. Add 50 µL of Stop Solution to each well.

14. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

** NOTES:
* Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

**The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.

Quick Guide: Standard Calibration

The following table indicates the protein standard contained in the Uncoated kit is calibrated against NIBSC standards.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>ng of eB standard</th>
<th>ng of NIBSC standard</th>
<th>U of NIBSC standard</th>
<th>NIBSC Lot #</th>
</tr>
</thead>
<tbody>
<tr>
<td>hIL-2</td>
<td>1</td>
<td>1.1</td>
<td>14.6</td>
<td>86/564</td>
</tr>
<tr>
<td>hIL-4</td>
<td>1</td>
<td>2.2</td>
<td>22</td>
<td>88/656</td>
</tr>
<tr>
<td>hIL-5</td>
<td>1</td>
<td>2.2</td>
<td>22</td>
<td>90/586</td>
</tr>
<tr>
<td>hIL-6</td>
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<td>1.7</td>
<td>170</td>
<td>89/548</td>
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<tr>
<td>hIL-8</td>
<td>1</td>
<td>1.8</td>
<td>180</td>
<td>89/520</td>
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<tr>
<td>hIL-10</td>
<td>1</td>
<td>0.8</td>
<td>4</td>
<td>93/722</td>
</tr>
<tr>
<td>hIL-12</td>
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<td>0.8</td>
<td>8</td>
<td>95/544</td>
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<tr>
<td>hIL-17A</td>
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<td>0.9</td>
<td>9000</td>
<td>01/420</td>
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<tr>
<td>hIFN-g</td>
<td>1</td>
<td>1.1</td>
<td>22</td>
<td>87/586</td>
</tr>
<tr>
<td>hTNF-a</td>
<td>1</td>
<td>0.9</td>
<td>36</td>
<td>87/650</td>
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<tr>
<td>mIL-2</td>
<td>1</td>
<td>3.1</td>
<td>310</td>
<td>93/566</td>
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<tr>
<td>mIL-4</td>
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<td>mIL-6</td>
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<td>8.5</td>
<td>850</td>
<td>93/730</td>
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<tr>
<td>mIFN-g*</td>
<td>1</td>
<td>4.5</td>
<td>Gg02-901-533</td>
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<tr>
<td>mTNF-a</td>
<td>1</td>
<td>1.7</td>
<td>340</td>
<td>88/532</td>
</tr>
</tbody>
</table>

* Mouse IFN-g is calibrated using NIH standard [Lot Gg02-901-533] and is measured in Units (U)
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possibility</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. High background</td>
<td>1. Improper and inefficient washing</td>
<td>1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as</td>
</tr>
<tr>
<td></td>
<td>2. Cross contamination from other specimens or positive control</td>
<td>2. Repeat ELISA being careful when washing and pipetting</td>
</tr>
<tr>
<td></td>
<td>3. Contaminated substrate</td>
<td>3. Substrate should be colorless. Replace</td>
</tr>
<tr>
<td></td>
<td>4. Incorrect dilutions, e.g., conjugate concentration was too high</td>
<td>4. Repeat using correct dilutions</td>
</tr>
<tr>
<td>B. No signal</td>
<td>1. Improper, low protein binding capacity plates were used</td>
<td>1. Repeat ELISA using recommended high binding capacity plates</td>
</tr>
<tr>
<td></td>
<td>2. Wrong substrate was used</td>
<td>2. Repeat ELISA using the correct substrate</td>
</tr>
<tr>
<td></td>
<td>3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity</td>
<td>3. Repeat ELISA making no enzyme inhibitor is present in any buffers.</td>
</tr>
<tr>
<td></td>
<td>4. Coated capture antibody in ELISA/ELISPOT Diluent rather than Coating Buffer</td>
<td>4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody.</td>
</tr>
<tr>
<td>C. Very weak signal</td>
<td>1. Improper and inefficient washing</td>
<td>1. Make sure washing procedure is done correctly, with a soak time.</td>
</tr>
<tr>
<td></td>
<td>2. Incorrect dilutions of standard</td>
<td>2. Follow recommendations of standard preparation exactly as written on the C of A</td>
</tr>
<tr>
<td></td>
<td>3. Insufficient incubation time</td>
<td>3. Repeat ELISA following the protocol carefully for each step</td>
</tr>
<tr>
<td></td>
<td>4. Incorrect storage of reagents</td>
<td>4. Store reagents at the correct temperature as indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards.</td>
</tr>
<tr>
<td></td>
<td>5. Wrong filter in ELISA reader was used</td>
<td>5. Use the correct wavelength setting</td>
</tr>
<tr>
<td></td>
<td>6. Wrong plate used</td>
<td>6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates</td>
</tr>
<tr>
<td>D. Variation amongst replicates</td>
<td>1. Improper and inefficient washing</td>
<td>1. Make sure washing procedure is done correctly; see C of A. Edge effects can be avoided by moving samples and standards in from the</td>
</tr>
<tr>
<td></td>
<td>2. Poor mixing of samples</td>
<td>2. Mix samples and reagents gently and equilibrate to proper temperature</td>
</tr>
<tr>
<td></td>
<td>3. Plates not clean</td>
<td>3. Plates should be wiped on bottom before measuring absorbance</td>
</tr>
<tr>
<td></td>
<td>4. Reagents have expired</td>
<td>4. Order a new Ready-Set-Go ELISA.</td>
</tr>
</tbody>
</table>
Documentation and support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty
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