Human TGF-β2 Immunoassay

Catalog Number: EA800102

For the quantitative determination of human Transforming growth factor-beta 2 (TGF-β2) concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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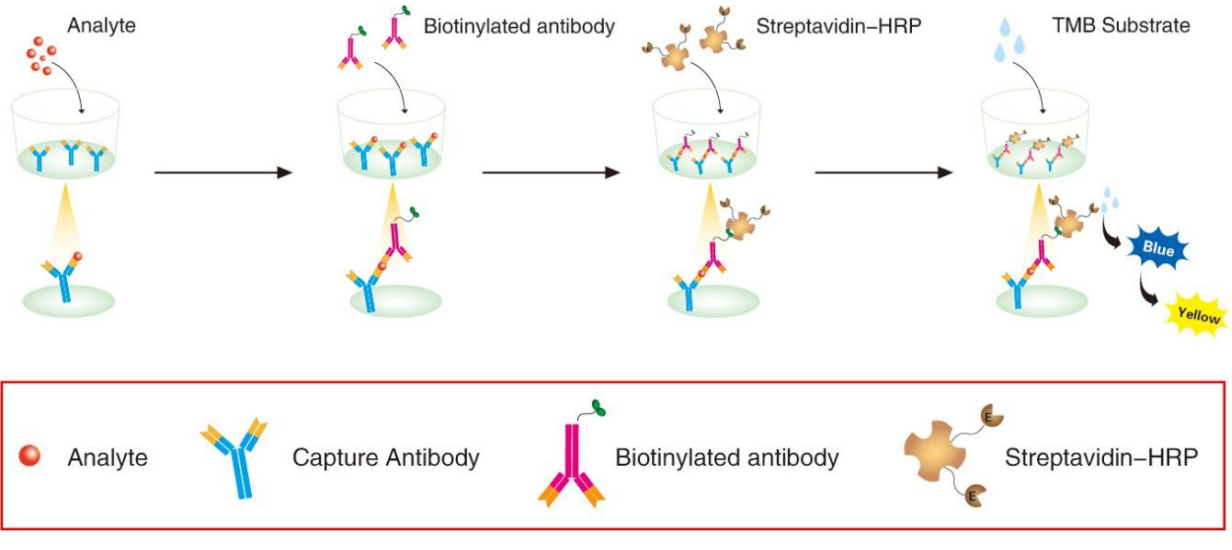
BACKGROUND

Vascular endothelial growth factor (TGF-β2)TGF-β2 (Transforming growth factor-beta 2) is a secreted protein known as a cytokine that performs many cellular functions and has a vital role during embryonic development. It is an extracellular glycosylated protein. It is known to suppress the effects of interleukin dependent T-cell tumors. TGFB1, TGFB2, and TGFB3 all function through the same receptor signaling systems. They are members of the large TGF-β superfamily. TGF-β proteins are highly pleiotropic cytokines that regulate processes such as immune function, proliferation and epithelial mesenchymal transition. TGF-β activation from latency is controlled both spatially and temporally, by multiple pathways that include actions of proteases such as plasmin and MMP9, and/or by thrombospondin 1 or selected integrins. The active form of TGF-β2 is a disulfide-linked homodimer which is generated by activation of the latent TGF-β2 complex. TGF-βs are multifunctional cytokines that activate or repress multiple genes in a wide variety of cell types. Depending on the cell context, TGF-β can control many cellular processes including growth, morphogenesis, and apoptosis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF-β2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TGF-β2 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for TGF-β2 is added to detect the captured TGF-β2 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



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1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized,2200 pg/ml upon reconstitution	3 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C** for six months
20 x Assay Buffer	1 bottle	Store at 2-8°C** for six months
Reagent Diluent	1 bottle	Store at 2-8°C** for six months
20 x PBS	1 bottle	Store at 2-8°C** for six months
1 M HCl	1 bottle	Store at 2-8°C** for six months
Neutralization Buffer	1 bottle	Store at 2-8°C** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C** for six months
Substrate Solution	1 bottle	Store at 2-8°C** for six months
Stop Solution	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.
6. Human TGF-β2 controls (optional; available from Solarbio).

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **20 x PBS** - pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
4. **20 x Assay Buffer** - 20 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
5. **Standard\Specimen (3 vials)** - human TGF-β2 Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The unreconstituted standard can be stored at 4℃ for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 μL of 1 x Assay Buffer to a Standard vial to make the high standard concentration of 2200 pg/mL and vortex 30 sec. A seven-point standard curve is generated using 2-fold serial dilutions in 1 x Assay Buffer, vortex 20 sec for each of dilution step.

   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

6. **Working solution of Biotin-Conjugate anti-human TGF-β2 antibody(1 vial)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS to a vial and vortex 30 sec. If the entire 96-well plate is used, take 200 µL of detection antibody to 10.5 mL of Reagent Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20℃ until use.

   ***The working solution should be used within one day after dilution.**

7. **Working solution of Streptavidin-HRP(53µL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 53 µL HRP Conjugate sufficient for a 96-well plate. If the volume is less than 53µL, add sterile 1 x PBS to reach 53 µL and vortex 10 sec. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of Reagent Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4℃ for up to 3 months. DO NOT FREEZE.

   ***The working solution should be used within one day after dilution.**

Activation of TGF-β2 in Biological Specimens

Biological specimens such as plasma need to be activated prior to TGF-β2 immunoassay.

Materials: 1 N HCl, Neutralization Buffer

Procedure

1. Add 25 µl of 1 N HCl to 50 µl of biological specimen (such as plasma) and mix well.
2. Incubate 10 min at room temperature.
3. Add 25 µl of Neutralization Buffer to neutralize the acidified sample and mix well.
4. Assay immediately. It may be a good start point if the activated sample is diluted 3-fold with 1 x Washer buffer.

Note: The activated specimens need to be diluted with 1 x Assay Buffer if its OD450 reading exceeds the upper limit of the standard curve and the dilution factor can be up to 20 folds depending on the TGF-β2 density.

ASSAY PROCEDURE

Prepare all reagents and standards as directed.

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Add 100µl standard or samples to each well, incubate 60 minutes,room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human TGF-β2 antibody to each well, incubate 60 minutes,room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,room temperature(25±2℃).

⇩ Aspirate and wash 5 times

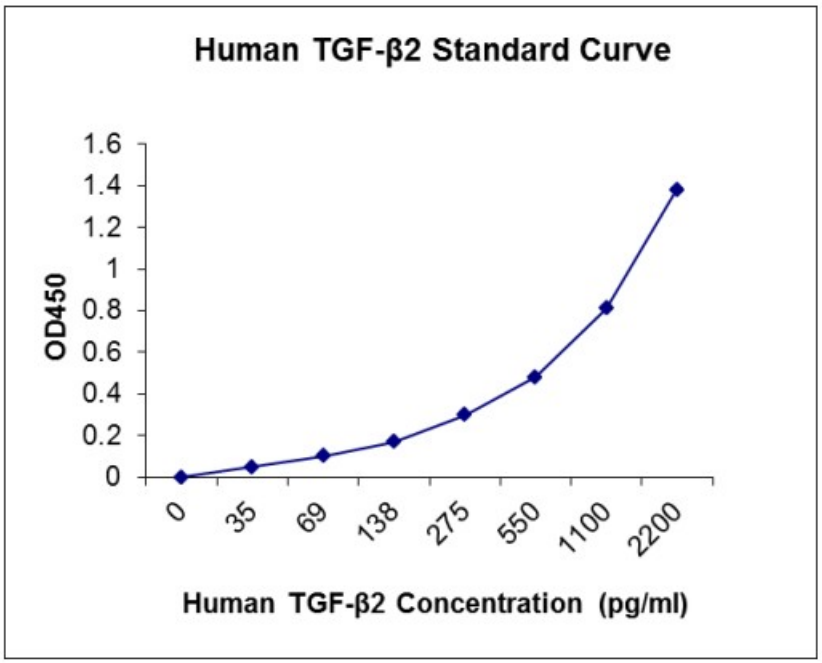
Add 100µl Substrate solution to each well, incubate 10-20 minutes (depending on signal),room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the TGF-β2 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Representative standard curve for TGF-β2 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 18 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human TGF-β2. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
ApoAI	IL-1ra	BMP1
Adiponectin	IL-2	BMP2
BMP1	IL-3	BMP4
BMP2	IL-5	MMP-9
BMP3	TGF-β2	TGF-β2
BMP4	TNF-α	
TGF-β1		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of TGF-β2 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of TGF-β2 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	88	81-95
Cell culture supernatants	96	92-100

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of TGF-β2 in various matrices and diluted with the appropriate Sample Diluent to

produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	85	92
	Range (%)	81-89	86-98
1:4	Average% of Expected	90	98
	Range (%)	87-93	94-102

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