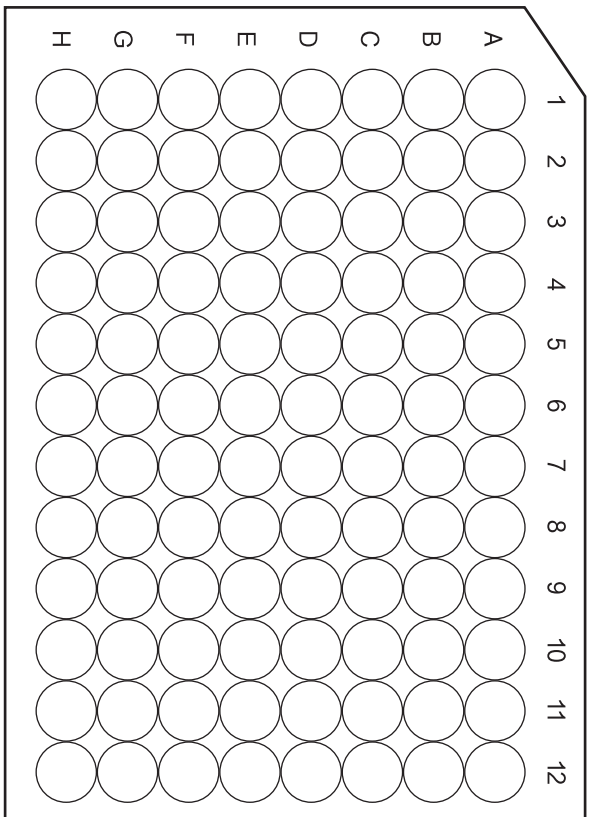

DESCRIPTION

Mouse TGF-β2 Immunoassay

Catalog Number: SEKM-0036

For the quantitative determination of mouse TGF-β2 concentrations in cell culture supernates, serum, and plasma.

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

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

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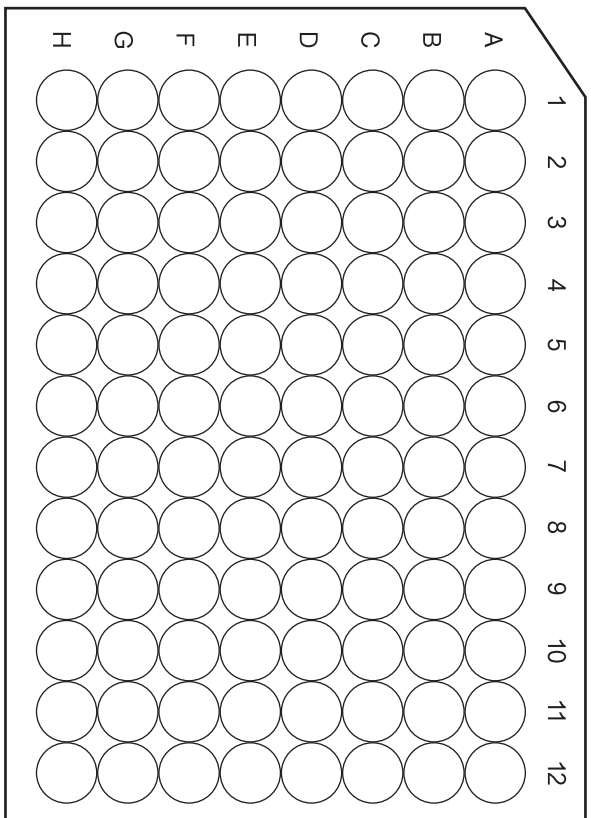

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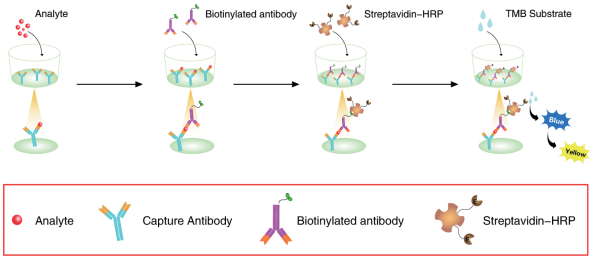
BACKGROUND

TGF-β2 (Transforming Growth Factor beta 2) is one of three closely related mammalian members of the large TGF-β superfamily that share a characteristic cysteine knot structure . TGF-β1, 2 and 3 are encoded by separate genes, but are often called isoforms. They are highly pleiotropic cytokines that are proposed to act as cellular switches regulating processes such as immune function, proliferation and epithelial-mesenchymal transition. Mammalian TGF-β2 is secreted as a 395 amino acid (aa) proprotein that is processed by a furin-like convertase to generate an N-terminal latency-associated peptide (LAP, ~232 aa) and a C-terminal mature TGF-β2 (~112 aa) that remain associated via hydrogen bonding. Mature mouse and rat TGF-β2 share 100% aa sequence identity, and share 97% aa identity with human, porcine, canine, equine and bovine TGF-β2.

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-β 2 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.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio 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.

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	93	105
	Range (%)	86-103	96-115
1:4	Average% of Expected	94	103
	Range (%)	82-105	91-118
1:8	Average% of Expected	90	99
	Range (%)	83-101	91-105
1:16	Average% of Expected	95	102
	Range (%)	90-103	90-113

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 2 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse TGF-β2 . The factors listed below were prepared at 100ng/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 mouse	Recombinant rat	Recombinant human
Activin A		Activin RIA
Activin RIB		Activin RIIA
Activin RIIB		BMP-2
TGF-β1		BMP-4
TGF-β RI		Follistatin
TGF-β RII		Inhibin A
TGF-β RIII		Inhibin B
		Latent TGF-β2
		LTBP-1
		LTBP-2

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.

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	93	88-98
Cell culture supernatants	96	92-100

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℃**
Standard - lyophilized,500 pg/ml upon reconstitution	2 vials	Store at 2-8°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(40X) - 300 ul/vial	1 vial	Store at 2-8°C **for six months
Standard/Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
plate cover seals	4 pieces	
1N HCL	1 vial	Store at 2-8°C **for six months
1N NaoH	1 vial	Store at 2-8°C **for six months

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

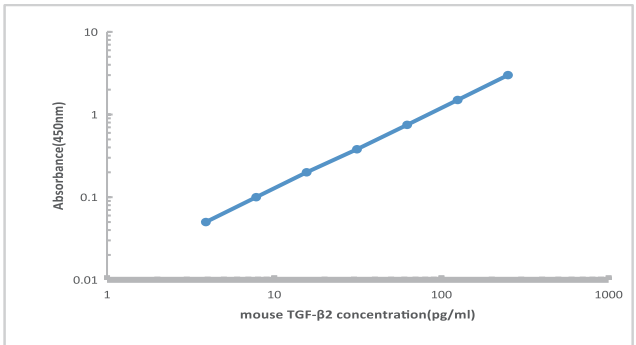
1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

SAMPLE 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 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: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and sample to room temperature (20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of 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.



Representative standard curve for TGF-β2 ELISA.

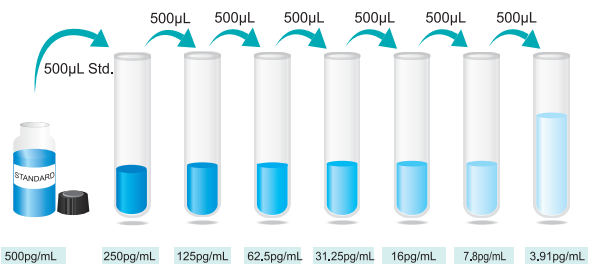
CALCULATION OF RESULTS

1.The standard curve is used to determine the amount of samples.
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.

Typical data using the TGF-β2 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.023	0.016	0.019	-
3.91	0.248	0.246	0.247	0.228
7.8	0.361	0.358	0.359	0.340
16	0.536	0.532	0.534	0.515
31.25	0.852	0.845	0.849	0.829
62.5	1.384	1.373	1.378	1.359
125	2.253	2.235	2.244	2.225
250	3.671	3.641	3.656	3.637

3.**Standard/Sample -** Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/ Sample Diluent into 250pg/ml tube and the pipette 500uL remaining tubes. Use the stock solution of 500pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 250 pg/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).



4.**Working solution of Biotin-**Conjugate anti-mouse TGF-β2 antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
***The working solution should be used within one day after dilution.**
5.Working solution of Streptavidin-HRP: Make a 1:40 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
***The working solution should be used within one day after dilution.**
Sample Pretreatment
Activation methods of serum or plasma samples:
1). Add 5 μserum or plasma samples into 225 μl standard / sample diluent.

2) Add 10 μl 1 N HCL, cover the tube and mix thoroughly, incubate for 60 ±2 minutes at2-8 ℃.

3) Add 10 μl1 N NaOH, cover the tube and mix thoroughly (total volume 250 μ l, sample was diluted by 1: 50)

4) Use it immediately or store at -20/-70 ℃ for 3 days. The final result should be multiplied by the dilution factor.

(Note: the level of TGF- β2 in different sample may be quite different, please control the dilution flexibly according to the actual situation).

Activation method of cell culture supernatant sample:

1) Add 100 μl specimen into 80 μstandard / specimen diluent.

2) Add 10 μ l 1N HCL, cover the tube and mix thoroughly, incubate for 60 ±2 minutes at 2-8 ℃.

3) Add 10 μl1 N NaOH, cover the tube and mix thoroughly (total volume 200 μ l, sample was diluted by 1: 2)

4) Use it immediately or store at -20/-70 ℃ for 3 days. The final result should be multiplied by the dilution factor.

(Note: the level of TGF- β2 in different sample may be quite different, please control the dilution flexibly according to the actual situation).

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-mouse TGF-β2 antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 15 minutes,37℃. Protect from light.

⇩

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