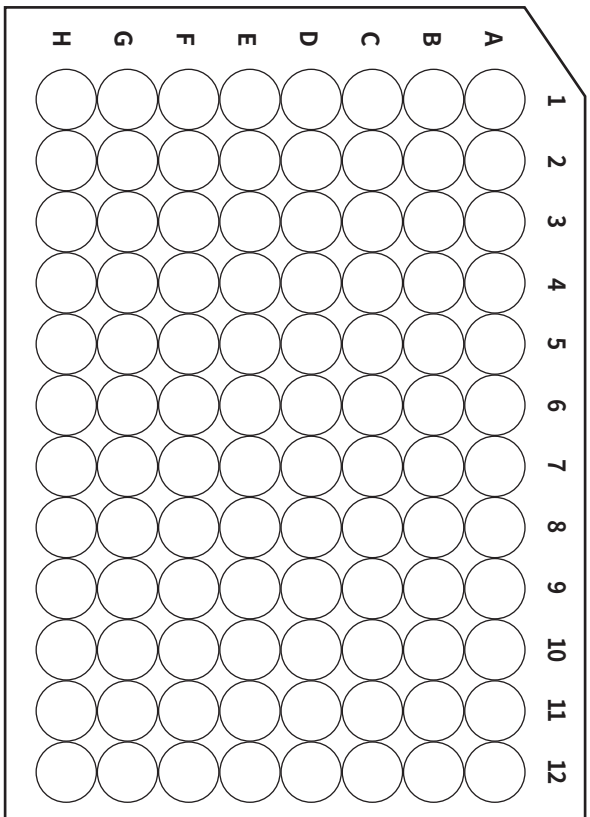

DESCRIPTION

Mouse TGF-β1 Immunoassay

Catalog Number: SEKM-0035

For the quantitative determination of mouse TGF-β1 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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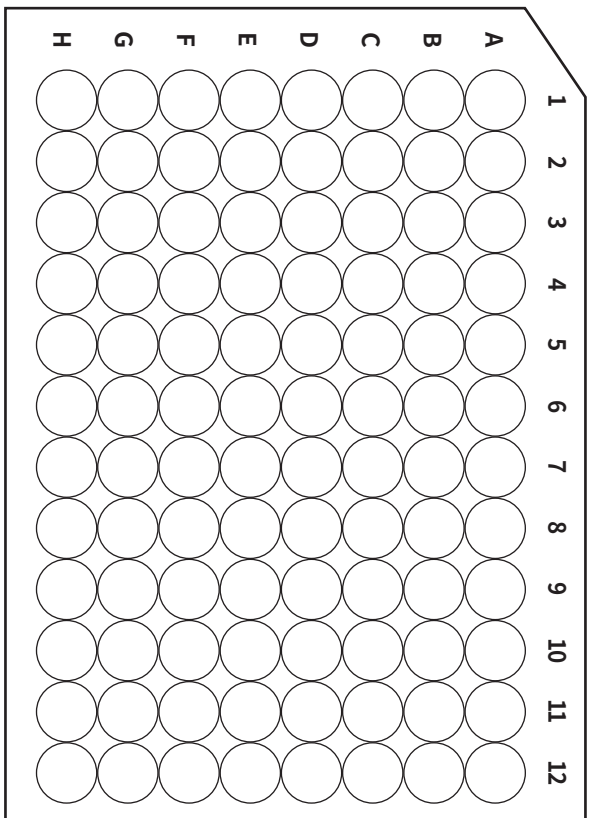

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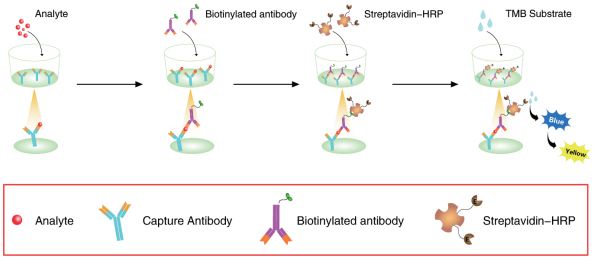
BACKGROUND

ITransforming growth factor beta (TGF-β) proteins (including the three closely related mammalian isoforms TGF-β1, -2 and -3) are pleiotropic cytokines that regulate extracellular matrix production, wound healing, immune functions, cell proliferation and differentiation. They belong to the large TGF-β superfamily, which also includes the activins/inhibins/MIS, bone morphogenetic proteins (BMPs), growth / differentiation factors (GDFs), Lefty1 and 2, and the distantly related GDNF family of neurotrophic factors. All family members show a characteristic cysteine-knot that is formed from multiple intrachain disulfide bonds. The mouse, rat, porcine and canine TGF-β1 cDNAs encode a 390 amino acid (aa) residue precursor that contains a 29 aa signal peptide and a 361 aa pro-protein. The pro-protein for each species is proteolytically processed via a furin-like convertase to generate an N-terminal 249 aa latency-associated peptide (LAP), and a C-terminal 112 aa mature TGF-β1. Both LAP and mature TGF-β1 exist as disulfide-linked homodimers.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF-β1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TGF-β1 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for TGF-β 1 is added to detect the captured TGF-β1 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-β1 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	88	101
	Range (%)	85-91	95-107
1:4	Average% of Expected	91	105
	Range (%)	87-95	101-109
1:8	Average% of Expected	92	103
	Range (%)	83-102	98-112
1:16	Average% of Expected	104	99
	Range (%)	96-117	90-107

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse TGF-β1 . 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
TGF-βRI/Fc Chimera		TGF-β1
		TGF-β2
		TGF-β3

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

RECOVERY: The recovery of TGF-β1 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,2000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°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(100X) - 120 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.

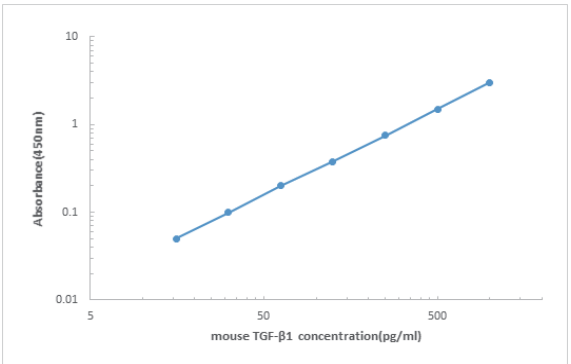
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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: TIt 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-β1 ELISA.

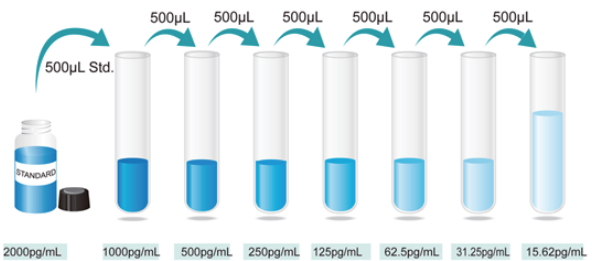
CALCULATION OF RESULTS

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-β1 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-β1 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.041	0.045	0.043	----
15.62	0.114	0.118	0.116	0.073
31.25	0.171	0.173	0.172	0.129
62.5	0.323	0.327	0.325	0.282
125	0.596	0.592	0.594	0.551
250	1.115	1.118	1.117	1.074
500	1.954	1.952	1.953	1.910
1000	3.113	3.115	3.114	3.071

3.**Standard/Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000 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 1000pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 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-β1 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 5.**
5.Working solution of Streptavidin-HRP: Make a 1:100 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.**
Activation methods of serum or plasma samples:
1). In 225 μl standard / sample diluent, 5 μl serum or plasma samples were added.
2) Add 10 μl 1 N HCL, to cover and mix up and down for 60 ±2 minutes at

2-8 ℃.

3) Add 10 μl 1 N NAOH, to cover and mix well up and down. (total volume 250 μl , 50 times dilution)

4) It can be stored at 70 ℃ for 3 days. The calculation result should be multiplied by the dilution multiple. (note: the level of TGF- β1 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)In 80μl standard / sample diluent, 100 μl sample was added.

2) Add 10 μ l 1N HCL, to cover and mix up and down for 60 ±2 minutes at 2-8 ℃.

3) Add 10 μ l 1N NaOH, to cover and mix well up and down. (the total volume is 200 μ l, that is, twice the dilution).

4) It can be stored at 70 ℃ for 3 days. The calculation result should be multiplied by the dilution multiple.. (note: the levels of TGF- β 1 in different sample may vary greatly. 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-β1 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.