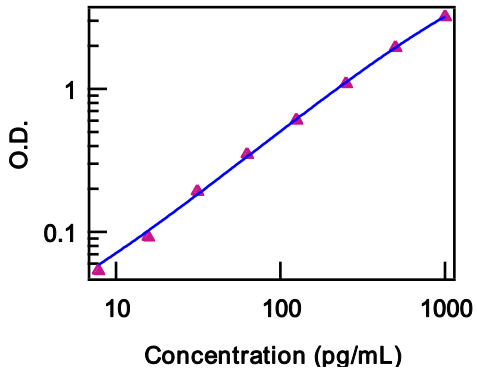
Human/Mouse TGF beta 1 ELISA Ready-SET-Go! (2nd Generation)

Catalog Number: 88-8350
Also known as: Transforming Growth Factor beta 1, TGF-b1
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Human/Mouse TGF beta 1 ELISA Ready-SET-Go!

Product Information

Contents: Human/Mouse TGF beta 1 ELISA Ready-SET-Go! (2nd Generation)
REF **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.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

This Human/Mouse TGF beta 1 Ready-SET-Go! 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 differntiation of CD4+CD25+ Treg cells from peripheral CD4+CD25- progeny. TGF beta 1-induced regulatory T cells have been termed iTreg.

Components

Capture Antibody. Pre-titrated, purified antibody

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

Human/Mouse TGF beta 1 ELISA Ready-SET-Go! (2nd Generation)

Catalog Number: 88-8350
Also known as: Transforming Growth Factor beta 1, TGF-b1
RUO: For Research Use Only. Not for use in diagnostic procedures.

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
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards.
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 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 occuring, 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.

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 occuring 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
Fantini MC, Becker C, Tubbe I, Nikolaev A, Lehr HA, Galle P, Neurath MF. TGF-beta – induced Foxp3+ regulatory T cells suppress Th1-mediated experimental colitis. Gut. 2006 May;55(5):671-80.

Peng Y, Laouar Y, Li MO, Green EA, Flavell RA. TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. Proc Natl Acad Sci U S A. 2004 Mar 30;101(13):4572-7.

Roberts AB, Sporn MB. Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta).

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

Human/Mouse TGF beta 1 ELISA Ready-SET-Go! (2nd Generation)

Catalog Number: 88-8350
Also known as: Transforming Growth Factor beta 1, TGF-b1
RUO: For Research Use Only. Not for use in diagnostic procedures.

Growth Factors. 1993;8(1):1-9.

Miyazono K, Heldin CH. Structure, function and possible clinical application of transforming growth factor-beta. J Dermatol. 1992 Nov;19(11):644-7

Related Products
00-4970 Cell Stimulation Cocktail (500X)
BMS249/2INST* Human TGF-beta 1 Instant ELISA
BMS249/3* Human TGF-beta1 Platinum ELISA
BMS8249FF* Human TGF-beta1 FlowCytomix Simplex
BMS8608FF* Mouse TGF-beta1 FlowCytomix Simplex

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

TDS Protocol

Research Use Only

Other Materials Needed

- Solutions for activating samples (not needed for standards)
  - 1 N HCl
  - 1 N NaOH
- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp flat-bottom)
  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 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

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

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written. All other set components should be stored at 2-8°C.

Time Requirements

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

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

TDS Protocol

Research Use Only

Experimental Procedure

1. Coat Corning Costar 9018 or NUNC Maxisorp 96 well ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer* (diluted to 1X). 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 concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Acid Activation of Samples: To activate latent TGF-ß1 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 TGF-ß1, so controls should be run to determine baseline concentrations of TGF-ß1 in culture media.
    1. 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.]
    2. Serum or plasma: Dilute 1:5 in PBS*, then treat as above for supernatants.
6. Using Assay 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 acid-activated samples to the appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
7. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
8. Add 100 µl/well of detection antibody diluted in 1X Assay 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 5 washes.
10. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay 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 7 washes.
12. Add 100 µl/well of Substrate 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.

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

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

TDS Protocol

Research Use Only

Ready-SET-Go Cytokine ELISA Set Buffers:

- Assay Diluent (5X concentrate): Dilute 1/5 in DI water.
- Substrate Solution: Ready to use (1X); 100 µl per well.

Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were	1. Repeat ELISA, using recommended high binding

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

TDS Protocol

Research Use Only

	used	capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor
C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each step□s incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the □frost free□ freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com