Human TGF-β1
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

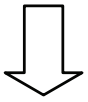
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

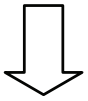
Thank you for choosing Assaypro.

Assay Summary

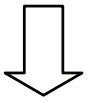
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



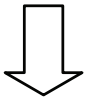
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 2 hours.



Wash, then add 50 µl of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 25 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
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2								
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AssayMax Human Transforming Growth Factor-β1 (TGF-β1) ELISA Kit

Catalog No. ET3102-1
Sample Insert/Reference Only

Introduction

Transforming growth factor-β1 (TGF-β1) is one of the transforming growth factor beta (TGF-β) family cytokines and exerts pleiotropic effects upon a wide variety of cell types. TGF-β1 has been demonstrated to be of fundamental importance in the development, physiology, and pathology of the vascular system (1). It is known to maintain a balance between apoptosis and cellular dysfunction (2). Over-expression of TGF-β1 is the cellular change associated with abnormal extracellular matrix deposition in nodular glomerulosclerosis (3) and may be a pathogenetic mechanism in tumor progression (4). High serum levels of TGF-β1 probably mirror an anti-inflammatory response, which might play a role in controlling the systemic immune response (5).

Principle of the Assay

The AssayMax Human TGF-β1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of TGF-β1 in cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TGF-β1 in less than 5 hours. A murine monoclonal antibody specific for human TGF-β1 has been pre-coated onto a 96-well microplate with removable strips. TGF-β1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human TGF-β1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**

- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human TGF-β1 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against TGF-β1.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human TGF-β1 Standard:** Human TGF-β1 in a buffered protein base (4 ng, lyophilized).
- **Biotinylated Human TGF-β1 Antibody (80x):** A 80-fold concentrated biotinylated polyclonal antibody against TGF-β1 (100 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.

- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 4 ng of Human TGF-β1 Standard with 2 ml of EIA Diluent to generate a 2 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the TGF-β1 standard solution twofold with equal volume of EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.063, and 0.031 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[TGF-β1] (ng/ml)
P1	Standard (2 ng/ml)	2.000
P2	1 part P1 + 1 part EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.031
P8	EIA Diluent	0.000

- **Biotinylated Human TGF-β1 Antibody (80x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Human TGF-β1 Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human TGF-β1 Antibody to each well and incubate for 2 hours.
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for approximately 25 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

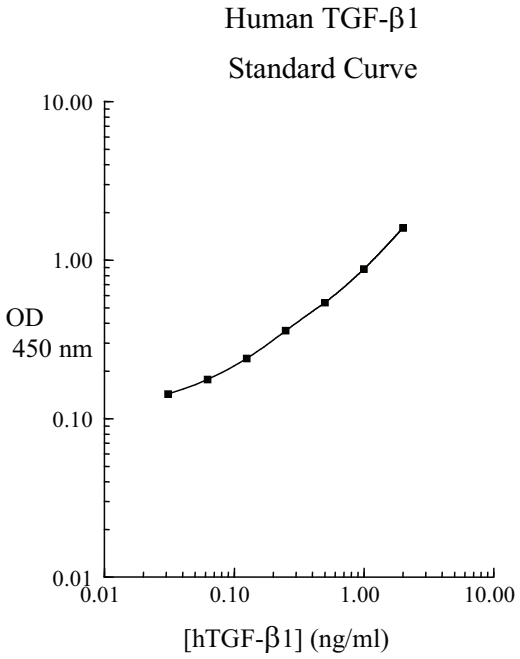
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of TGF-β1 is typically ~ 0.03 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.1% respectively.
- This assay recognizes both natural and recombinant human TGF-β1.

References

(1) Ghosh J *et. al.* (2005) *Cardiovasc Pathol.* 14(1): 28-36
(2) Jacob T *et. al*. (2005) *J Vasc Surg*. 41(3): 523-30
(3) Zhao HL *et. al*. (2004) *Am J Kidney Dis*. 44(6): 1039-49
(4) Maluccio M *et. al*. (2003) *Transplantation.* 76(3): 597-602
(5) Widhe M *et. al.* (2002) *Immunology.* 107(1): 46-55

Version 3.9