

Human Transforming Growth Factor-beta 1 (TGF- β 1) ELISA Kit

Introduction

Transforming growth factor-beta 1 (TGF- β 1) is one of the transforming growth factor beta (TGF- β) family cytokines and exerts pleiotropic effects upon a wide variety of cell types. TGF- β 1 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- β 1 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- β 1 probably mirror an anti-inflammatory response, which might play a role in controlling the systemic immune response (5).

Principal of the Assay

The Human TGF- β 1 ELISA kit is designed for detection of TGF- β 1 in cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TGF- β 1 in 4.5 hours. A murine monoclonal antibody specific for human TGF- β 1 has been pre-coated onto a microplate. TGF- β 1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human TGF- β 1, 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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **TGF- β 1 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against TGF- β 1.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **TGF- β 1 Standard:** Human TGF- β 1 in a buffered protein base (2 ng, lyophilized).

- **Biotinylated TGF- β 1 Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against TGF- β 1 (80 μ l).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **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

- Store kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along with zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 2 ng of human TGF- β 1 Standard with 1 ml of EIA Diluent to generate a solution of 2 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the TGF- β 1 standard solution twofold with equal volume of EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625 and 0.031 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20⁰C.

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

- **Biotinylated TGF- β 1 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C .
- **Wash Buffer Concentrate (20x):** 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 ($20\text{-}30^{\circ}\text{C}$).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 μl of Biotinylated TGF- β 1 Antibody to each well and incubate for two hours.
- Wash five times with 200 μl of Wash Buffer as 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 five times with 200 μl of Wash Buffer as above.
- Add 50 μl of Chromogen Substrate per well and incubate for approximately 15 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**.

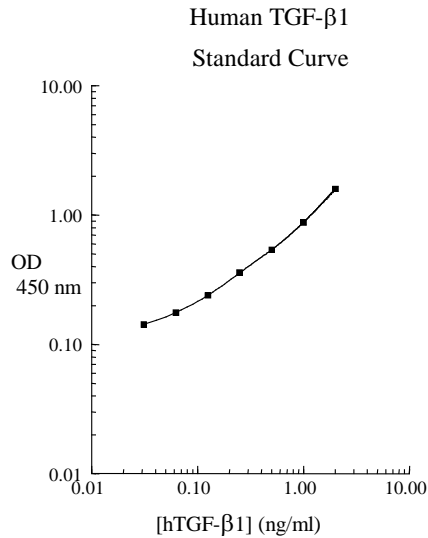
Data Analysis

- Calculate the mean value of the 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- β 1 is typically < 30 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.2% and 7.0% respectively.
- This assay recognizes both natural and recombinant human TGF- β 1.

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

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