Human LAP TGF-β1 ELISA Kit

Assaypro LLC

30 Triad South Drive

St. Charles, MO 63304

T (636) 447-9175

F (636) 447-9475

www.assaypro.com

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/samples 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 per well.
Incubate 30 minutes.

⇩

Wash, then add 50 μl of
Chromogen Substrate per well.
Incubate 20 minutes.

⇩

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

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Assay Template

AssayMax Human Latency Associated Peptide (LAP) TGF-β1 ELISA Kit
Catalog No. ET3103-1
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 LAP TGF-β1 ELISA kit is designed for detection of LAP TGF-β1 in plasma, serum, milk, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures LAP TGF-β1 in less than 5 hours. A murine monoclonal antibody specific for human LAP TGF-β1 has been pre-coated onto a microplate. LAP TGF-β1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human LAP 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 LAP TGF-β1 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against LAP 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 LAP TGF-β1 Standard:** Human LAP TGF-β1 in a buffered protein base (2 ng, lyophilized).
- **Biotinylated Human LAP TGF-β1 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against LAP TGF-β1 (140 μl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 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

- Store components of the kit at 2-8°C or -20°C upon arrival 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 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month 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.
- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and use supernatants. Dilute samples 1:2 with MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. The samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 2 ng of Human LAP TGF-β1 Standard with 1 ml of MIX 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 duplicate or triplicate standard points by serially diluting the LAP TGF-β1 standard solution twofold with equal volume of MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.063 and 0.031 ng/ml solutions. MIX 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	[LAP TGF-β1] (ng/ml)
P1	Standard (2 ng/ml)	2.000
P2	1 part P1 + 1 part MIX Diluent	1.000
P3	1 part P2 + 1 part MIX Diluent	0.500
P4	1 part P3 + 1 part MIX Diluent	0.250
P5	1 part P4 + 1 part MIX Diluent	0.125
P6	1 part P5 + 1 part MIX Diluent	0.063
P7	1 part P6 + 1 part MIX Diluent	0.031
P8	MIX Diluent	0.000

- **Biotinylated Human LAP TGF-β1 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 MIX 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 (20-30°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 LAP TGF-β1 Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample 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 LAP 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 20 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.

H. LAP TGF-β1 Standard Curve

OD 450 nm vs [h. LAP TGF-β1] (ng/ml)
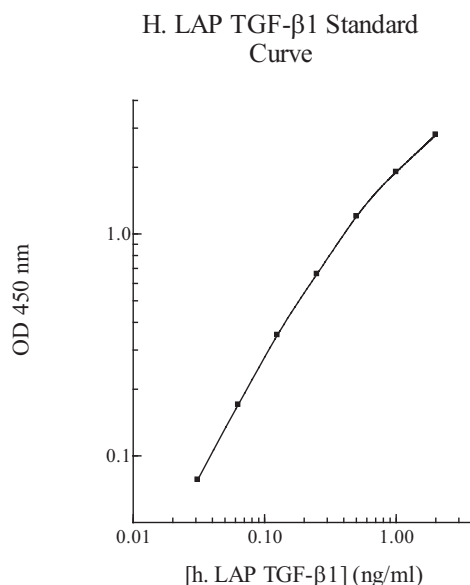
Performance Characteristics

- The minimum detectable dose of LAP TGF-β1 is typically ~ 0.03 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.2% respectively.
- This assay recognizes both natural and recombinant human LAP TGF-β1.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
No Dilution	89%	92%
1:2	99%	98%
1:4	104%	101%

Recovery

Standard Added Value	5 – 150 ng/ml
Recovery %	84 – 109 %
Average Recovery %	99 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 50%
Mouse	None
Rat	None
Swine	None
Human	100%

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 1.0