# Human TGF beta 1 (LAP) ELISA Kit

Enzyme Immunoassay for the quantification of Human TGF beta 1 (LAP) in Human Serum, plasma (EDTA, heparin) and cell culture supernatants.

Catalog number: ARG82777

Package: 96 wells

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## INTRODUCTION

Background: This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate a latency-associated peptide (LAP) and a mature peptide, and is found in either a latent form composed of a mature peptide homodimer, a LAP homodimer, and a latent TGF-beta binding protein, or in an active form consisting solely of the mature peptide homodimer. The mature peptide may also form heterodimers with other TGFB family members. This encoded protein regulates cell proliferation, differentiation and growth, and can modulate expression and activation of other growth factors including interferon gamma and tumor necrosis factor alpha. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease. [provided by RefSeq, Aug 2016]

Function: Transforming growth factor beta-1 proprotein: Precursor of the Latency-associated peptide (LAP) and Transforming growth factor beta-1 (TGF-beta-1) chains, which constitute the regulatory and active subunit of TGF-beta-1, respectively.

[Latency-associated peptide]: Required to maintain the Transforming growth factor beta-1 (TGF-beta-1) chain in a latent state during storage in extracellular matrix (PubMed:28117447). Associates non-covalently with TGF-beta-1 and regulates its activation via interaction with 'milieu molecules', such as LTBP1, LRRC32/GARP and LRRC33/NRROS, that control activation of TGF-beta-1

(PubMed:2022183, PubMed:8617200, PubMed:8939931, PubMed:19750484, PubMed:22278742, PubMed:19651619). Interaction with LRRC33/NRROS regulates activation of TGF-beta-1 in macrophages and microglia (Probable). Interaction with LRRC32/GARP controls activation of TGF-beta-1 on the surface of activated regulatory T-cells (Tregs) (PubMed:19750484, PubMed:22278742, PubMed:19651619). Interaction with integrins (ITGAV:ITGB6 or ITGAV:ITGB8) results in distortion of the Latency-associated peptide chain and subsequent release of the active TGF-beta-1 (PubMed:22278742, PubMed:28117447).

Transforming growth factor beta-1: Multifunctional protein that regulates the growth and differentiation of various cell types and is involved in various processes, such as normal development, immune function, microglia function and responses to neurodegeneration (By similarity). Activation into mature form follows different steps: following cleavage of the proprotein in the Golgi apparatus, Latency-associated peptide (LAP) and Transforming growth factor beta-1 (TGF-beta-1) chains remain non-covalently linked rendering TGF-beta-1 inactive during storage in extracellular matrix (PubMed:29109152). At the same time, LAP chain interacts with 'milieu molecules', such as LTBP1, LRRC32/GARP and LRRC33/NRROS that control activation of TGF-beta-1 and maintain it in a latent state during storage in extracellular milieus (PubMed:2022183, PubMed:8617200, PubMed:8939931, PubMed:19750484, PubMed:22278742, PubMed:19651619). TGF-beta-1 is released from LAP by integrins (ITGAV:ITGB6 or ITGAV:ITGB8): integrin-binding to LAP stabilizes an alternative conformation of the LAP bowtie tail and results in distortion of the LAP chain and subsequent release of the active TGF-beta-1 (PubMed:22278742, PubMed:28117447). Once activated following release of

LAP, TGF-beta-1 acts by binding to TGF-beta receptors (TGFBR1 and TGFBR2), which transduce signal (PubMed:20207738). While expressed by many cells types, TGF-beta-1 only has a very localized range of action within cell environment thanks to fine regulation of its activation by Latency-associated peptide chain (LAP) and 'milieu molecules' (By similarity). Plays an important role in bone remodeling: acts as a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts (By similarity). Can promote either T-helper 17 cells (Th17) or regulatory T-cells (Treg) lineage differentiation in a concentration-dependent manner (By similarity). At high concentrations, leads to FOXP3-mediated suppression of RORC and down-regulation of IL-17 expression, favoring Treg cell development (By similarity). At low concentrations in concert with IL-6 and IL-21, leads to expression of the IL-17 and IL-23 receptors, favoring differentiation to Th17 cells (By similarity). Stimulates sustained production of collagen through the activation of CREB3L1 by regulated intramembrane proteolysis (RIP) (PubMed:25310401). Mediates SMAD2/3 activation by inducing its phosphorylation and subsequent translocation to the nucleus (PubMed:25893292, PubMed:29483653, PubMed:30696809). Can induce epithelial-to-mesenchymal transition (EMT) and cell migration in various cell types (PubMed:25893292, PubMed:30696809). [UniProt]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TGF beta 1 (LAP) has been pre-coated onto a microtiter

plate. Standards or samples are pipetted into the wells and any TGF beta 1 (LAP) present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TGF beta 1 (LAP) is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of TGF beta 1 (LAP) bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of TGF beta 1 (LAP) in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit at 4°C for short-term storage or at-20°C for long-term storage.
Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 8 X 12 strips | 4°C. Unused strips should be sealed tightly in the air-tight pouch. |
| Standard (Lyophilized) | 2 X 10 ng/Vial | 4°C |
| Standard/Sample diluent | 30 ml (Ready to use) | 4°C |
| Antibody conjugate concentrate (100X) | 1 vial (100 µl) | 4°C |
| Antibody diluent buffer | 12 ml (Ready to use) | 4°C |

| | | |
|---|---|---|
| HRP-Streptavidin concentrate (100X) | 1 vial (100 µl) | 4°C |
| HRP-Streptavidin diluent buffer | 12 ml (Ready to use) | 4°C |
| 25X Wash Buffer | 20 ml | 4°C |
| TMB substrate | 10 ml (Ready to use) | 4°C (Protect from light) |
| STOP solution | 10 ml (Ready to use) | 4°C |
| Plate sealer | 4 strips | Room temperature |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. The kit can also be stored at-20°C for long-term storage.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent

before using.

- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 25X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for few minutes before use.
- Samples contain azide cannot be assayed.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot &

store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

## REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 25 X wash buffer with deionized water to yield 1X wash buffer. The pH value of dissolved 1X wash buffer should between pH7.2 to pH7.6. The diluted 1X wash buffer is stable for a week at 2°C to 8°C.
- **1X Antibody conjugate**: It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation. Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- **1X HRP-Streptavidin Solution**: It is recommended to prepare this reagent

immediately prior to use and use it within 1 hours after preparation. Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)

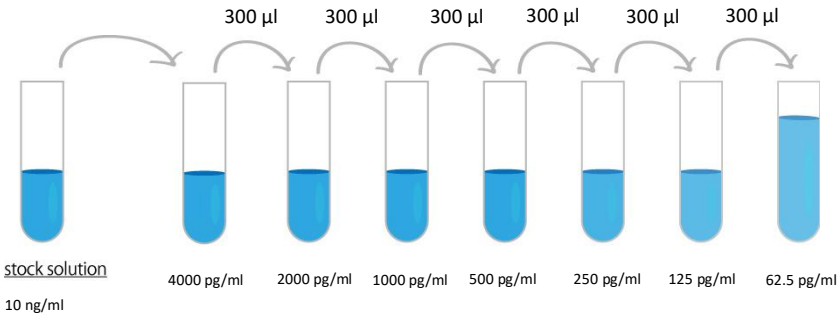
- **Sample**: If the initial assay found samples contain TGF beta 1 (LAP) higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

  **(It is recommended to do pre-test to determine the suitable dilution factor)**.

- **Standards**: Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml** of **Standard/Sample diluent** to yield a stock concentration of **10 ng/ml**. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.

  **Note:** The reconstituted standard solutions are best used within 2 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at -20°C for up to 48 hours. Avoid repeated freeze-thaw

cycles.




Dilute TGF beta 1 (LAP) standard as according to the table below:

| Standard | TGF beta 1 (LAP) Conc. (pg/ml) | µl of Standard/Sample diluent | µl of standard |
|---|---|---|---|
| S7 | 4000 pg/ml | 600 | 400 (10 ng/ml Stock) |
| S6 | 2000 pg/ml | 300 | 300 (S7) |
| S5 | 1000 pg/ml | 300 | 300 (S6) |
| S4 | 500 pg/ml | 300 | 300 (S5) |
| S3 | 250 pg/ml | 300 | 300 (S4) |
| S2 | 125 pg/ml | 300 | 300 (S3) |
| S1 | 62.5 pg/ml | 300 | 300 (S2) |
| S0 | 0 | 300 | 0 |

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate should be prewarm at 37°C few minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard TGF beta 1 (LAP) detection

curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of TGF beta 1 (LAP) amount in samples. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples and zero controls** (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes at 37°C.**
3. **Aspirate each well**. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time. Wash step is not necessary in this step.
4. Add **100 µl** of **1X Antibody conjugate** into each well, gently tap the plate to mix well. Cover wells and **incubate for 60 minutes at 37°C**.
5. Aspirate each well and wash, repeating the process two times for a total **three washes**. Wash by filling each well with **1X Wash Buffer** (or 0.01M PBS or TBS) (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well, gently tap the plate to mix well. Cover wells and **incubate for 30 minutes at 37°C**.
7. Aspirate each well and wash, repeating the process four times for a total **five washes**. Wash by filling each well with **1X Wash Buffer** (or 0.01M PBS or TBS) **(300 µl)** using a squirt bottle, manifold dispenser, or autowasher,

keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.

8. Add **90 µl** of **TMB substrate** to each well. Incubate for **15-30 minutes** at 37°C in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated TGF beta 1 (LAP) standard solutions; the other wells show no obvious color).
9. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at **450 nm** immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the

corresponding concentration from the standard curve.

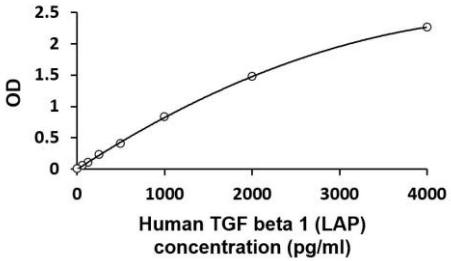
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



## QUALITY ASSURANCE

### Sensitivity

The minimum detectable dose (MDD) of Human TGF beta 1 (LAP) ranged from 62.5- 4000 pg/ml. The mean MDD was 32 pg/ml.

### Specificity

This assay recognizes natural and recombinant Human TGF beta 1 (LAP). No significant cross-reactivity or interference with the factors below was observed: There is no detectable cross-reactivity with other relevant proteins.

### Intra-assay and Inter-assay precision

The CV values of intra-assay was 6.4% and inter-assay was 6.8%.