



Human TGF beta 1 (Latent) ELISA Kit

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

Catalog number: ARG82653

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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 TGF-beta 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]

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,

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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 (TGFB1 and TGFB2),

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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 (Latent) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TGF beta 1 (Latent) present is bound by the immobilized antibody. After washing

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away any unbound substances, a biotin-conjugated antibody specific for TGF beta 1 (Latent) 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 (Latent) 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 \pm 2nm. The concentration of TGF beta 1 (Latent) in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
C2	Standard (Lyophilized)	3 X 50 pM/Vial	4°C
C3	Standard diluent buffer	20 ml (ready to use)	4°C
C4	Antibody conjugate concentrate	1 vial (400 µl)	4°C
C5	Antibody diluent buffer	16 ml (ready to use)	4°C
C6	HRP-Streptavidin concentrate	1 vial (400 µl)	4°C (Protect from light)
C7	HRP-Streptavidin diluent buffer	16 ml (ready to use)	4°C
C8	20X Wash buffer	50 ml	4°C
C9	TMB substrate	12 ml (ready to use)	4°C (Protect from light)
C10	STOP solution	12 ml (ready to use)	4°C
C11	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 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

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- Avoid using reagents from different batches.
- 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.

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 and 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

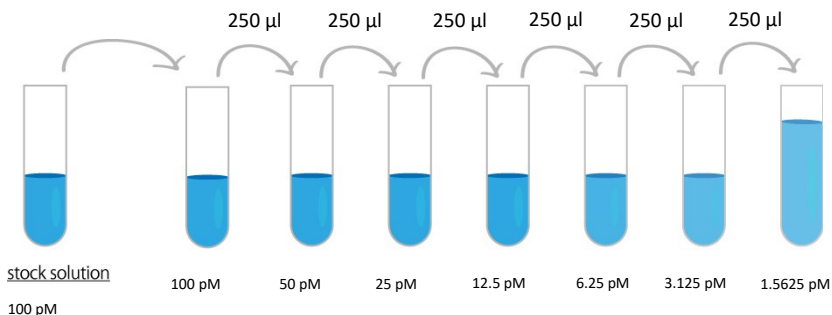
- **1X Wash buffer:** Dilute **20X** Wash buffer (#C8) into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water)
The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate:** 20 minutes before use, dilute **30X** antibody conjugate concentrate (#C4) into antibody diluent buffer (#C5) to yield 1X Detection antibody solution.
- **1X HRP-Streptavidin Solution:** 20 minutes before use, dilute **30X** HRP-Streptavidin concentrate solution (#C6) into HRP-Streptavidin diluent buffer (#C7) to yield 1X HRP-Streptavidin Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Sample:** If the initial assay found samples contain TGF beta 1 (Latent) higher than the highest standard, the samples can be diluted with Standard diluent buffer (#C3) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

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

- **Standards:** Reconstitute the standard (#C2) with **0.5 ml** standard diluent buffer (#C3) to yield a stock concentration of 100 pM. Keep the buffer in the vial for at least **15 min at RT** to make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pM), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 100 pM, 50 pM, 25 pM, 12.5 pM, 6.25 pM, 3.125 pM, 1.5625 pM.

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DO NOT reuse the reconstituted standard.



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

Standard	TGF beta 1 (Latent) Conc.	µl of Standard diluent	µl of standard
S7	100 pM	0	500 (100 pM Stock)
S6	50 pM	250	250 (S7)
S5	25 pM	250	250 (S6)
S4	12.5 pM	250	250 (S5)
S3	6.25 pM	250	250 (S4)
S2	3.125 pM	250	250 (S3)
S1	1.5625 pM	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min before use.

Standards, samples and controls 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 (standard diluent buffer) into wells, gently tap the plate to mix well. Incubate for **1.5 h** at

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37°C.

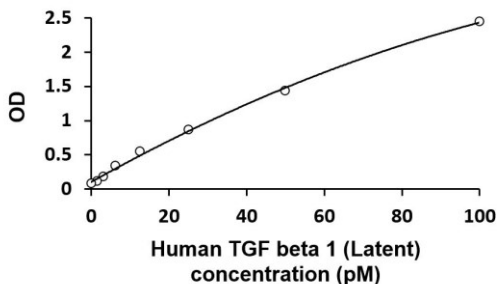
3. Aspirate each well and wash, repeating the process four times for a total **five washes**. Wash by filling each well with 1x Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for **1 hour at 37°C**.
5. Aspirate each well and **wash as step 3**.
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** in dark.
7. Aspirate each well and wash as step 3.
8. Add **100 µl** of TMB Reagent (#C9) to each well, gently tap the plate to mix well. Incubate for **15 minutes at 37°C** in dark.
9. Add **100 µl** of Stop Solution (#C10) to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm immediately**. It is recommended read the absorbance within 3 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using 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. 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 (Latent) ranged from 1.56- 100 pM. The mean MDD was 0.78 pM.

Specificity

This assay recognizes natural and recombinant Human TGF beta 1 (Latent). No significant cross-reactivity or interference with the factors below was observed: Not react with latent TGF beta 2 and latent TGF beta 3.

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.