Canine TGF beta 1 ELISA Kit

Enzyme Immunoassay for the quantification of Dog TGF beta 1 in Dog Plasma.

Catalog number: ARG82641

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

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

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 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]
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. 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). 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. 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. 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. Once activated following release of LAP, TGF-beta-1 acts by binding to TGF-beta receptors (TGFBR1 and TGFBR2), which transduce signal. 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). Mediates SMAD2/3 activation by inducing its phosphorylation and subsequent translocation to the nucleus. Can induce epithelial-to-mesenchymal transition (EMT) and cell migration in various cell types. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique. An antibody specific for TGF beta 1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TGF beta 1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TGF beta 1 is added to each well and incubate to bind to TGF beta 1 captured by the first antibody. 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 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 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.

Component	Quantity	Storage information
Antibody-coated microplate	1 X 96 well plate	4°C
Standard	3 X 1 ng/Vial (Lyophilized)	4°C
Biotin-antibody conjugate concentrate	1 vials (lyophilized)	4°C
HRP-Streptavidin conjugate concentrate	1 vial (53 µl)	4°C
Diluent buffer	21 ml (Ready to use)	4°C
1 N HCl	2.5 ml (Ready to use)	4°C
Neutralization Buffer	2.5 ml (Ready to use)	4°C
20X PBS	30 ml	4°C
20X Assay Buffer	20 ml	4°C
TMB substrate	10.5 ml (Ready to use)	4°C (Protect from light)
STOP solution	5.5 ml (Ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water

- Sterile 1 x PBS
- 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.
- 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.
- Briefly spin down (6000Xg for 1 min) the Standards, Biotin-antibody conjugate and HRP-streptavidin conjugate before use.
- If crystals are observed in the 20X Assay Buffer and sample diluent, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve.
- Brief vortex samples and diluted standards for 10 sec to mix well before add to the 96 well plate.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Do not let strips dry, as this will inactivate active components in wells.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be

assayed in duplicates.

- HRP Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
- The Stop Solution is an acid solution, handle with caution.
- 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.

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.

Activation of TGF beta 1 in Biological Specimens

Biological specimens such as plasma need to be activated prior to TGF beta 1 immunoassay.

Materials: 1 N HCl, Neutralization Buffer

Procedure

1. Add 25 µl of 1 N HCl to 50 µl of biological specimen (such as plasma) and mix well.

2. Incubate 10 min at room temperature.

3. Add 25 µl of Neutralization Buffer to neutralize the acidified sample and mix well.

4. Assay immediately. It may be a good start point if the activated sample is diluted 3-fold with 1 x Washer buffer.

Note: The activated specimens need to be diluted with 1 x Assay Buffer if its OD 450 reading exceeds the upper limit of the standard curve and the dilution factor can be up to 20 folds depending on the TGF beta 1 density.

REAGENT PREPARATION

- 1X PBS: Dilute 20X PBS into deionized distilled water to yield 1X PBS.
- 1X Assay Buffer: Dilute 20X Assay Buffer into 1X PBS to yield 1X Assay buffer. The diluted 1X Assay Buffer can be stored at 4°C.
- 1x Biotin-antibody Conjugate: The lyophilized Biotin-antibody conjugate could be stored at 4°C to -20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the Biotin-antibody Conjugate with 200 µl of sterile 1 x PBS, vortex for 30 sec and keep the antibody in the vail for 5 min to completely dissolve. Centrifuge the vial for 1 min at 6000 x g before opening. Aliquot and store the antibody stock at -20°C until use. Avoid repeated freeze-thaw cycles.

  If the entire 96-well plate is used, dilution of the 200 µl of concentrated Biotin-Conjugate solution with 10.5 ml Diluent Buffer to yield 1X Biotin-antibody Conjugate working solution.
- 1X HRP-streptavidin conjugate: Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. The stock vial includes 53 µl of HRP-streptavidin concentrate. Please confirm if the vial contains 53 µl of HRP-streptavidin concentrate before further dilution. If it is less

than 53 µl, add sterile 1X PBS to reach 53 µl and vortex briefly for 10 sec. Make a 1:200 dilution of the concentrated HRP-streptavidin solution with Diluent Buffer (If the entire 96-well plate is used, add 53 µl concentrated HRP-streptavidin solution into 10.5 ml Diluent Buffer and mix thoroughly prior to the assay). The rest of undiluted HRP-streptavidin Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

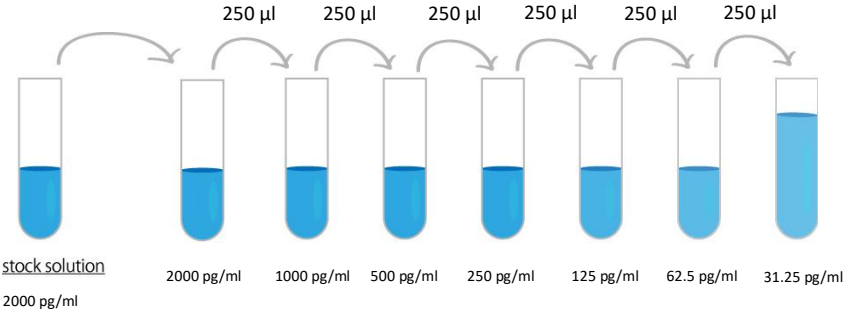
- Sample: If the initial assay found samples contain TGF beta 1 higher than the highest standard, the samples can be diluted with 1 x Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with 1 x Assay Buffer before assay.

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

- Standards: The non-reconstituted standard can be stored at 4°C or-20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the standard with 0.5 ml 1 x Assay Buffer to yield a stock concentration of 2000 pg/ml. Brief vortex the vials for 30 sec and keep the standard stock in the vail for 5 min to completely dissolve. Make sure the standard is dissolved completely and then centrifuge the vial for 1 min at 6000 x g before making serial dilutions. Aliquot and store the reconstituted standard at-20°C for up 2 days.

  The 1 x Assay Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with 1X Assay Buffer as according to the suggested concentration below: 2000 pg/ml, 1000

pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml. Brief vortex the vials for 30 sec for each standard dilution steps to mix well.



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

Standard	TGF beta 1 Conc. (pg/ml)	µl of 1X Assay Buffer	µl of standard
S7	2000 pg/ml	0	500 (2000 pg/ml Stock)
S6	1000 pg/ml	250	250 (S7)
S5	500 pg/ml	250	250 (S6)
S4	250 pg/ml	250	250 (S5)
S3	125 pg/ml	250	250 (S4)
S2	62.5 pg/ml	250	250 (S3)
S1	31.25 pg/ml	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be **assayed in duplicates.**

1. Lift the plate cover from the top left and cover the wells that are not used. Brief vortex and then spin down the standards and samples for 10 sec to mix completely before applying to the plate.
2. Add **100 µl** of standards, samples and zero controls (1X Assay Buffer) **in duplicates** into wells. Incubate for **1 hour** at **room temperature**.
3. Aspirate each well and wash, repeating the process twice for a **total three washes**. Wash by filling each well with **1× Assay Buffer** (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Assay Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **1x Biotin-antibody Conjugate working solution** to each well. Cover the plate and incubate **1 hour** at **room temperature**.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well. Cover wells and incubate for **20 minutes** at **room temperature** in dark.
7. Aspirate each well and **wash as step 3**.
8. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate for **10-20 minutes** (depending on signal) at **room temperature** in dark.
9. Add **50 µl** of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at **450 nm** immediately. (Optional:

it is recommended to detect background signal by reading the signal at 540-570 nm as reference wavelength).

CALCULATION OF RESULTS

1. (Optional) Subtract the absorbance of the value reading at 540-570 nm from the value reading at 450 nm.

2. Calculate the average absorbance values for each set of standards, controls and patient samples.

3. 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.

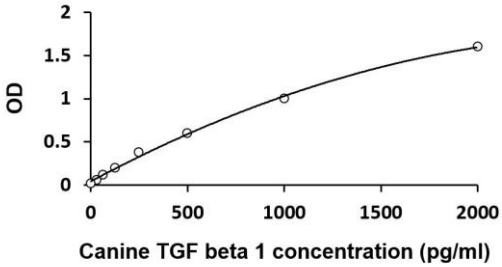
4. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

5. 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.

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 Canine TGF beta 1 ranged from 31-2000 pg/ml. The mean MDD was 16 pg/ml.

Specificity

This assay recognizes natural and recombinant Canine TGF beta 1. No significant cross-reactivity or interference with the factors below was observed: Recombinant Canine Adiponectin, ApoAI, BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, CRP, CCL2, CCL4, CCL5, FGF acidic, IGF1, HGF, HSP27, IFN gamma, IL1 alpha, IL1 beta, IL1RA, IL1RI, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL17C, IL21, MMP2, MMP9, PDGF, PLA2G7, prolactin, serpin E1, TGF beta 2, TGF beta 3, TLR1, TLR2, TLR3, TNF alpha, TNF RI, TNF RII, sIL2R, sIL6R, VEGF and VEGF R1.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 7% and inter-assay was 10%.