Tgfb1 ELISA Kit (Dog) (OKCD00149)

Instructions for use

For the quantitative measurement of Tgfb1 in serum, platelet-poor plasma, tissue homogenates, cell culture supernates and other biological fluids

Lot to lot variations can occur. Refer to the manual provided along with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Tgfb1 ELISA Kit (Dog) (OKCD00149) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for Tgfb1 has been pre-coated onto a 96-wellplate (12 x 8 Well Strips) and blocked. Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for Tgfb1 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample Tgfb1 captured in well.

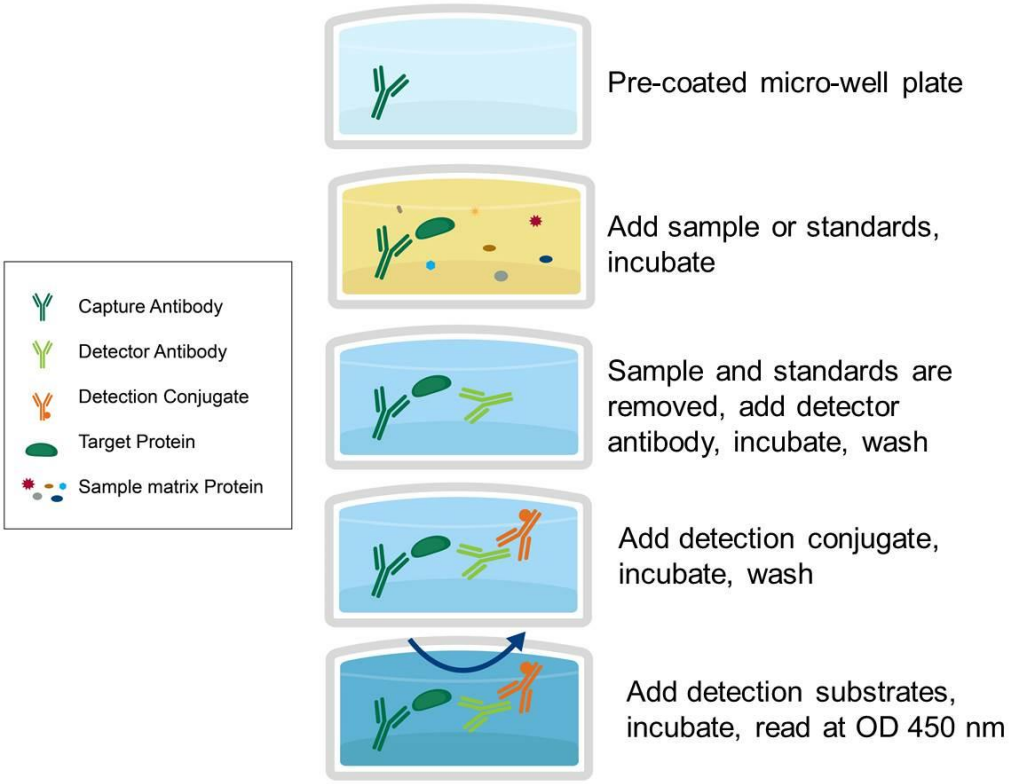
Background

Multifunctional protein that controls proliferation, differentiation and other functions in many cell types. Many cells synthesize Tgfb1 and have specific receptors for it. It positively and negatively regulates many other growth factors. It plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts. Can promote either T-helper 17 cells (Th17) or regulatory T-cells (Treg) lineage differentiation in a concentration-dependent manner. At high concentrations, leads to FOXP3-mediated suppression of RORC and down-regulation of IL-17 expression, favoring Treg cell development. 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. 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

General Specifications

General Specifications	
Range	15.62-1,000 pg/mL
LOD	< 5.6 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Dog Transforming growth factor beta-1 UniProt ID: P54831 GeneID: 403998 Target Alias: TGF-B1; CED; DPD1; LAP; Camurati-Engelmann Disease; Latency-associated peptide
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months or -20°C for long term storage (exceptions noted below). Avoid any freeze/thaw cycles.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-Tgfb1 Microplate	96 Wells (12 x 8 Well strips)	4°C for 1 Months -20°C for 6 Months
Tgfb1 Lyophilized Standard	2 x 1 ng	
100X Biotinylated Tgfb1 Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	Store at 4°C for 6 Months
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 6 mL	
TMB Substrate	1 x 9 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

• 12M HCl

• 10M NaOH

• HEPES

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

8.1 Dog Tgfb1 Assay Standards

8.1.1 Prepare the Tgfb1 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided 1 ng **Lyophilized Tgfb1 Standard** for each experiment. Prepare the stock 1,000 pg/mL Standard by reconstituting one tube of 1 ng **Lyophilized Tgfb1 Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Standard Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of seven serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Use the undiluted 1,000 pg/mL **Tgfb1 Standard** as the high standard point (Tube #1).

8.1.3.3 Add 300 µL of **Standard Diluent** to Tube #'s 2 – 8.

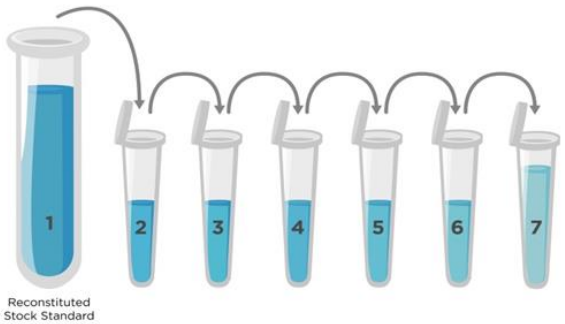
8.1.3.4 Prepare **Standard #2** by adding 300 µL of 1,000 pg/mL **Tgfb1** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	1,000 pg/mL Reconstituted Tgfb1 Standard	NA	NA	1,000	1,000 pg/mL
2	1,000 pg/mL	300	300	600	500 pg/mL
3	500 pg/mL	300	300	600	250 pg/mL
4	250 pg/mL	300	300	600	125 pg/mL
5	125 pg/mL	300	300	600	62.5 pg/mL
6	62.5 pg/mL	300	300	600	31.2 pg/mL
7	31.2 pg/mL	300	300	600	15.6 pg/mL
8	NA	0	300	300	0.0 (Blank)



8.2 1X Biotinylated Tgfb1 Detector Antibody

8.2.1 Prepare the **1X Biotinylated Tgfb1 Detector Antibody** immediately prior to use by diluting the **100X Biotinylated Tgfb1 Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated Tgfb1 Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X HRP-Avidin Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Wash Buffer

8.4.1 If crystals have formed in the **30X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.4.2 Add the entire 20 mL contents of the **30X Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 1M HCl

8.5.1 Add 8.33 mL of 12M HCl to 91.67 mL ultrapure water.

8.5.2 Mix gently and thoroughly.

8.6 1.2M NaOH/0.5M HEPES

8.6.1 Add 12 mL of 10M NaOH to 75 mL of ultrapure water. Mix gently and thoroughly.

8.6.2 Add 11.9 g of HEPES. Mix gently and thoroughly.

8.6.3 Bring the total volume to 100 mL using ultrapure water.

8.6.4 Mix gently and thoroughly.

8.7 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at °4C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Samples not indicated in the manual must be tested to determine if the kit is valid.
- Prepare samples as follows:

  - **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

  - **Platelet-poor plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. It is recommended to centrifuge samples for 10 minutes at 10,000 x g for complete platelet removal. Remove plasma and assay(see activation procedure) immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

  - **Tissue Homogenates** – Rinse 100 mg tissue with 1X PBS then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes then centrifuge for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

  - **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Dilute samples using **Standard Diluent**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

9.3 Latent TGFB1 Activation

• Ensure that samples after neutralization is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.

• Do not freeze activated samples.

• The solutions may be stored in polypropylene bottles at room temperature for up to one month.

• Wear protective clothing and safety glasses during preparation or use of these reagents.

• Do not activate the kit standards.

9.3.1 Serum/Plasma

9.3.1.1 Add 10 µL of **1M HCl** to 50 µL of serum/plasma

9.3.1.2 Incubate for 10 minutes are room temperature.

9.3.1.3 Neutralize the acidified solution by adding 10 µL of **1.2M NaOH/0.5M HEPES**.

9.3.1.4 Mix gently and thoroughly.

9.4.1.5 Add 80 µL of **Sample Diluent**. Assay immediately.

9.4.1.6 The final dilution factor is 3 which must be multiplied by the calculated measurement from regression of the standard curve.

9.3.2 Cell Culture Supernatants

9.3.2.1 Add 20 µL of **1M HCl** to 100 µL of cell culture supernatant.

9.3.2.2 Incubate for 10 minutes are room temperature.

9.3.2.3 Neutralize the acidified solution by adding 20 µL of **1.2M NaOH/0.5M HEPES**.

9.3.2.4 Mix gently and thoroughly.

9.4.2.5 Assay immediately.

9.4.2.6 The final dilution factor is 1.4 which must be multiplied by the calculated measurement from regression of the standard curve.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-Tgfb1 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate lid and incubate at 37°C for 60 minutes.

10.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated Tgfb1 Detector Antibody** to each well.

10.7 Cover with the well-plate lid and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 1 minute.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1XAvidin-HRP Conjugate** into each well and incubate at 37°C for 30 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 90 µL of **TMB Substrate** to each well and incubate at 37°C **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.17 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The **Tgfb1** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Reproducibility

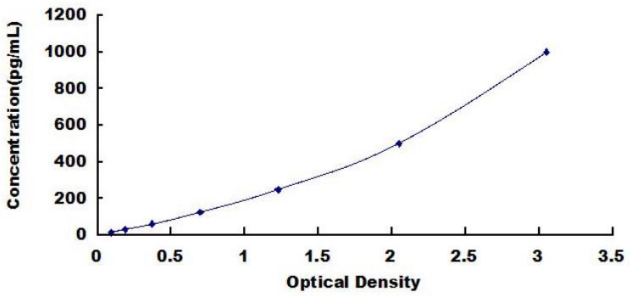
Intra-assay Precision: 3 samples with known low, middle and high levels Tgfb1 were tested with 20 replicates on one plate, respectively.

Inter-assay Precision: 3 samples with known low, middle and high level Tgfb1 were tested on 3 different plates, 8 replicates in each plate.

Mean Intra-Assay: CV ≤10%
Mean Inter-Assay: CV ≤12%

12.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Linearity

Kit linearity evaluated by testing serially diluted samples containing known concentrations of Tgfb1. Results are expressed as the percentage of the expected concentration measurement.

Sample Type	Dilution Level			
	1:2	1:4	1:8	1:16
Serum (n=5)	91-99%	97-106%	78-99%	79-93%
EDTA Plasma (n=5)	84-95%	79-101%	89-105%	87-99%
Heparin Plasma (n=5)	81-103%	83-97%	85-96%	80-90%

12.4 Recovery

The following matrices were spiked to known concentrations using recombinant Tgfb1. Recovery is expressed as the percentage of the expected concentration measurement.

Sample Type	Recovery Range (%)	Average (%)
Serum (n=5)	82-103	92
EDTA Plasma (n=5)	80-90	85
Heparin Plasma (n=5)	85-99	94

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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