# Mouse TGF beta1 ELISA Kit

Enzyme Immunoassay for the quantification of Mouse Transforming Growth Factor- beta 1 in serum, plasma, cell culture supernatants

Catalog number: ARG80211

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

TGF beta (Transforming Growth Factor beta) is a 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 (By similarity). Stimulates sustained production of collagen through the activation of CREB3L1 by regulated intramembrane proteolysis (RIP). 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 [provided by uniprot]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TGF beta1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TGF beta1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TGF beta1 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 beta1 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 beta1 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 | 8 X 12 strips | 4°C. Unused strips should be sealed tightly in the air-tight pouch. |
| Standard (Lyophilized) | 3 X 2 ng/vial | 4°C |
| Standard diluent buffer | 20 ml | 4°C |
| Antibody conjugate concentrate | 1 vial (400 µl) | 4°C |
| Antibody diluent buffer | 16 ml | 4°C |
| HRP-Streptavidin concentrate | 1 vial (400 µl) | 4°C (Protect from light) |
| HRP-Streptavidin diluent buffer | 16 ml | 4°C |
| 20X Wash buffer | 50 ml | 4°C |
| TMB substrate | 12 ml | 4°C (Protect from light) |
| STOP solution | 12 ml | 4°C |
| 1N HCl | 3 ml | 4°C |
| 1N NaOH | 3 ml | 4°C |
| Plate sealer | 6 strips | Room temperature |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 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.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- 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.
- 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 and aliquot & store samples at ≤ -20°C. 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. Remove serum and

assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Avoid using samples with hemolysis or hyperlipidemia. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

## Sample activation:

Cell Culture Supernatants

a. Add 100 µl sample into 80 µl Standard diluent buffer.
b. Add 10 µl 1N HCl, mix well and incubate for 60 ±2 min at 2-8°C.
c. Neutralize by adding 10 µl 1N NaOH, mix well.
d. Final predilution: 1:2.

Serum & Plasma

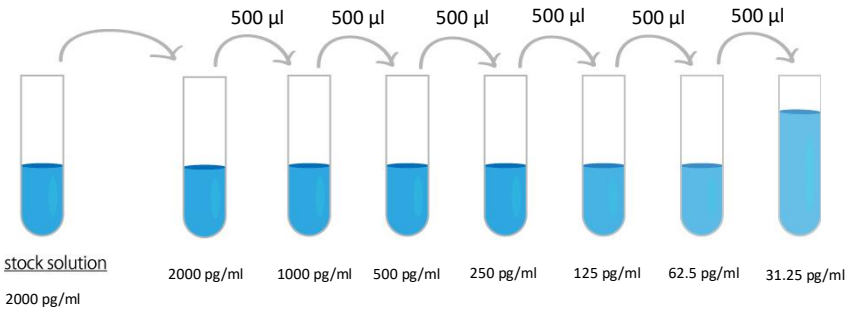
e. Add 5 µl sample into 225 µl Standard diluent buffer.
f. Add 10 µl 1N HCl, mix well and incubate for 60 ±2 min at 2-8°C.
g. Neutralize by adding 10 µl 1N NaOH, mix well.
h. Final predilution: 1:50.

* Use the activated sample immediately, or within 3 days when stored at -20/-70°C.

* Sample was diluted after the activation process, please pay attention to the predilution ratio when calculate the concentration of the target protein.

## REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X Antibody conjugate**: 20 min before use, dilute 30X antibody conjugate concentrate into 1X antibody diluent buffer to yield 1X Detection antibody solution.
- **1X HRP-Streptavidin Solution**: 20 min before use, dilute 30X HRP-Streptavidin concentrate solution into 1X HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- **Standards**: Reconstitute the standard with 1 ml standard diluent buffer to yield a stock concentration of 2000 pg/ml. Make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted 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.



## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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. Incubate for 1.5 h at 37°C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (350 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 Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X Antibody conjugate into each 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. Cover wells and incubate for 30 minutes at 37°C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450nm immediately.

## 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.

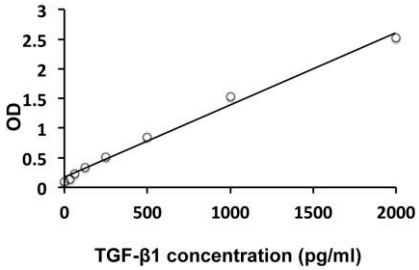
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. Sample was diluted after the activation process, please pay attention to the predilution ratio when calculate the concentration of the target protein.

## 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 Mouse TGF beta1 ranged from 31.25-2000 pg/ml. The mean MDD was 15 pg/ml.

## Specificity

This assay recognizes natural and recombinant Mouse TGF beta1. No significant cross-reactivity or interference with the factors below was observed: Human TGF- RI/Fc Chimera TGF-2; amphibian TGF-5

## Intra-assay and Inter-assay precision

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

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***For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.***

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.