MOUSE TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE TGF-β1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	MOUSE TGF-β1 ELISA
Catalog No.	SK00033-05
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	3.9 pg/mL
Sample Volume	100 µL
Sample Type	EDTA Plasma, Serum and Cell Culture Supernates
Sample Dilution	Plasma or Serum: Optimal dilutions should be determined by each laboratory for each application
Sample Pretreatment	Required
Specificity	Rat, Mouse, Human TGF-β1
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8 °C

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Mouse TGF-β1 immunoassay is a solid phase ELISA designed to measure TGF-β1 in cell culture supernates, serum and EDTA plasma. It contains recombinant TGF-β1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant TGF-β1. Results obtained with naturally occurring TGF-β1 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural TGF-β1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TGF-β1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TGF-β1 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for TGF-β1 is added to the wells. Following a wash to remove any unbound antibody reagent, Avidin-HRP Conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of TGF-β1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
TGF-β1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against TGF-β1.	**033-05-01**	**1 plate**
TGF-β1 Standard – 2000 pg/vial of recombinant TGF-β1 in a buffered protein base with preservative; lyophilized.	**033-05-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of an antibody against TGF-β1 with preservative; lyophilized.	**033-05-03**	**1 vial**
Positive Control - one vial of recombinant TGF-β1; lyophilized.	**033-05-04**	**1 vial**
Avidin-HRP Conjugate – 50 µl/vial, 250-fold concentrated solution of Avidin conjugate to HRP.	**AVHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB07**	**1 bottle**
Sample Pretreatment Solution A - 6 mL of activation buffer.	**PTS06**	**1 bottle**
Sample Pretreatment Solution B – 6 mL of neutralization buffer.	**PTS07**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C

or -70 °C for up to one month. Avidin-HRP Conjugate 250-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and 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, heparin, or citrate as an anticoagulant. 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION (BEFORE ACTIVATION PROCEDURE)

Tissue Culture Supernatants do not need to be diluted. *Animal serum used in culture media may contain high levels of latent TGF-β1, so controls should be run to determine baseline concentrations of TGF-β1 in culture media.

Serum and plasma samples may not need to be diluted, but if the sample absorbances are higher than the maximum standard, then a 2-fold dilution or higher dilution may be needed. A suggested 2-fold dilution is 125 µL sample + 125 µL **1X PBS**. **Optimal dilutions should be determined by each laboratory for each application with sample pretest.**

Use polypropylene test tubes.

ACTIVATION PROCEDURE

All samples require activation of latent TGF-β1 to the immunoreactive form before assay performance. **DO NOT ACTIVATE THE STANDARD OR POSITIVE CONTROL.**

1. To 100 µL sample add 20 µL **Sample Pretreatment Solution A**. Mix well.
2. Incubate 10 minutes at room temperature.
3. Add 20 µL of **Sample Pretreatment Solution B**. Mix well and assay within two hours.
Note: 1) Sample results must be multiplied by the dilution factor, 1.4 (from activation procedure). If samples generate values higher than the highest standard, further dilute the samples before activation with **1X PBS** and repeat the assay. 2) Do not activate the standard/positive control as it already contains active TGF-β1. 3) It is also possible that some serum and plasma samples may contain low levels of immunoreactive TGF-β1 that has disassociated from LAP. 4) Naturally occurring, free TGF-β1 may be measurable in this assay by evaluating samples without activation procedure.
Use polypropylene tubes.

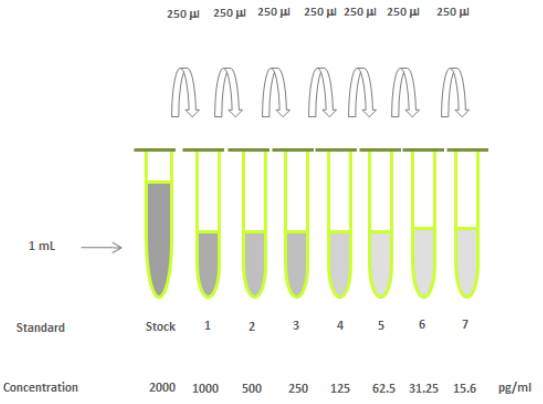
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

TGF-β1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **TGF-β1** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the hight standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml
# 7	250 µl of 6	250 µl	15.625 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Avidin-HRP Conjugate - Pipette 11.952 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 48 µL of 250-fold concentrated stock solution to prepare working solution. ***Note:*** *1x working solution of Avidin-HRP Conjugate should be used within a few days (protect from light).*

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. ***Note:*** *Positive Control could be reused within a few days if stored at -20 °C or -70 °C.*

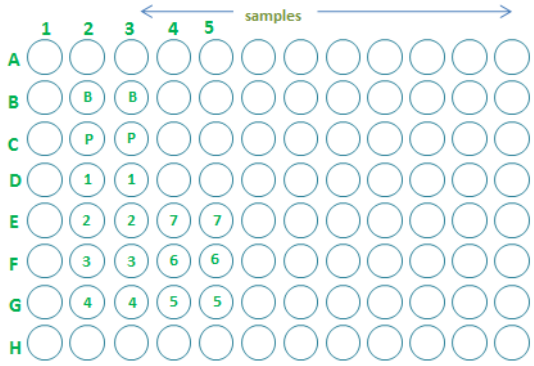
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **standard solutions** from #7 to #1 in reverse order of serial dilution (E4, E5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL **Avidin-HRP Conjugate working solution** to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 3-7 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the

color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TGF-β1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

The concentration read from the standard curve need to be multiplied by its dilution factor of 1.4 if samples were directly assayed after activation procedure. If samples required further dilution, then the concentration need to be multiplied by its dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant TGF-β1.

SENSITIVITY

The minimum detectable dose (MDD) of TGF-β1 was 3.9 pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

TGF-β1 STANDARD (PG/ML)	O.D. AT 450NM (CORRECTED)
Blank	0 (0.078)
15.6	0.076
31.2	0.197
62.5	0.344
125	0.674
250	1.298
500	2.299
1000	3.237

- **Lot:**
- **PC:**

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Mouse TGF-β1	100
Rat TGF-β1	100
Human TGF-β1	100
Human TGF-β2	0
Human LAP	0
Human TGF-β3	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of **standard, samples, positive control** to the well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Detection Antibody working solution** to each well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Avidin-HRP Conjugate working solution** to each well. Incubate 1 hour on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Substrate Solution** to each well. Incubate 3-7 min on plate shaker at RT. **Protect from light**.
↓
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min.