

RAT TRANSFORMING GROWTH FACTOR- β 1 (TGF- β 1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF TGF- β 1 CONCENTRATIONS IN CELL
CULTURE SUPERNATES, SERUM AND EDTA
PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

ELISA NAME	RAT TGF- β 1 ELISA
Catalog No.	SK00033-03
Lot No.	
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	3.9 pg/mL
Sample Volume	100 μ L
Sample Type	EDTA Plasma, Serum and Cell Culture Supernates
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application
Sample Pretreatment	Required
Specificity	Rat, Mouse, Human TGF- β 1
Calibration	TGF- β 1 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8 $^{\circ}$ C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Rat TGF- β 1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural TGF- β 1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant TGF- β 1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural TGF- β 1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for TGF- β 1. The capture antibody can bind to the TGF- β 1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against TGF- β 1 is added to the wells. After another washing of the plate, Avidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of TGF- β 1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix 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.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
TGF-β1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against TGF- β 1.	033-03-01	1 plate
TGF-β1 Standard – 2000 pg/vial of recombinant TGF- β 1 in a buffered protein base with preservative; lyophilized.	033-03-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of a biotinylated antibody against TGF- β 1 with preservative; lyophilized.	033-03-03	1 vial
Positive Control - one vial of recombinant TGF- β 1; lyophilized.	033-03-04	1 vial
Avidin-HRP Conjugate – 50 μ L/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) solution 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.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ 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- β 1, so controls should be run to determine baseline concentrations of TGF- β 1 in culture media.

Serum and plasma samples may not need to be diluted, but if the sample absorbance is higher than the maximum standard, then a 2-fold dilution or higher dilution may be needed. A suggested 2-fold dilution is 125 μ L sample + 125 μ L **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- β 1 to the immunoreactive form before assay performance. **DO NOT ACTIVATE THE STANDARD OR POSITIVE CONTROL.**

1. To 100 μ L sample add 20 μ L **Sample Pretreatment Solution A**. Mix well.
2. Incubate 10 minutes at room temperature.
3. Add 20 μ L 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- β 1. 3) It is also possible that some serum and plasma samples may contain low levels of immunoreactive TGF- β 1 that has disassociated from LAP. 4) Naturally occurring, free TGF- β 1 may be measurable in this assay by evaluating samples without activation procedure.

Use polypropylene tubes.

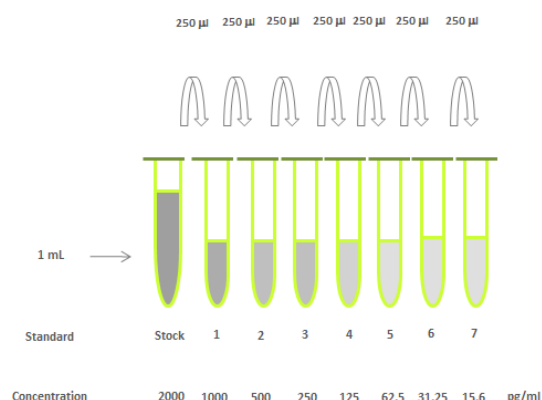
REAGENT PREPARATION

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- β 1 Standard - Reconstitute the TGF- β 1 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 μ L 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 high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

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



Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20°C or -70°C .

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 μ L 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).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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 μ L of **Dilution Buffer** to Blank wells.
4. Add 100 μ L of **Standard dilutions** from #7 to #1 in reverse order of serial dilution, **sample**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 μ L) 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 μ L 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 μ L **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 μ L of **Substrate Solution** to each well. Incubate for 2-5 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 μ L 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

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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 also.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Rat TGF- β 1	100
Human TGF- β 1	100
Mouse TGF- β 1	100
Human TGF- β 2	0
Human LAP	0
Human TGF- β 3	0









TYPICAL DATA

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

TGF- β 1 STANDARD (PG/ML)	O.D. AT 450NM (CORRECTED)
Blank	0 (0.114)
15.6	0.085
31.2	0.131
62.5	0.252
125	0.469
250	0.881
500	1.639
1000	2.525

- Lot:
- Positive Control:

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 μ l of standard dilutions, samples, or positive control to the well. Incubate 2 hours on plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ l Detection Antibody working solution to each well. Incubate 2 hours on plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ l 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 μ l Substrate Solution to each well. Incubate 2-5 min on plate shaker at RT. Protect from light.

Add 100 μ l Stop Solution to each well. Read 450nm within 15 min.