HUMAN TGF-β3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TGF-β3 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN TGF-β3 ELISA
Catalog No.	SK00058-01
Lot No.	
Formulation	96 T
Standard range	31-2000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	125 µl
Sample Type	Serum, EDTA Plasma and Cell Culture Supernates
Pretreatment	Require
Specificity	Human TGF-β3 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2 – 8°C

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INTRODUCTION

Human TGF-β3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human TGF-β3 in cell culture supernates, serum and EDTA plasma. It contains recombinant human TGF-β3 and antibodies raised against this protein. It has been shown to accurately quantitate recombinant human TGF-β3. Results obtained with naturally occurring TGF-β3 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 human TGF-β3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF-β3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TGF-β3 present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for TGF-β3 is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin-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-β3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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-β3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against TGF-β3.	**058-01-01**	**1 plate**
TGF-β3 Standard – 2000 pg/vial of recombinant human TGF-β3 in a buffered protein base with preservatives; lyophilized.	**058-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of polyclonal IgG Biotinylated against TGF-β3 with preservatives; lyophilized.	**058-01-03**	**1 vial**
Positive Control - one vial of recombinant human TGF-β3, lyophilized	**058-01-04**	**1 vial**
Streptavidin HRP Conjugate – 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	**DB01**	**2 bottles**
Sample Pretreatment Solution A - 5 mL of buffered protein based solution with preservatives	**PTS06**	**1 bottle**
Sample Pretreatment Solution B – 5 mL of buffered protein based solution with preservatives	**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**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 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 and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or – 70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

ACTIVATION PROCEDURE

All samples require activation of latent TGF-β3 to the immunoreactive form before assay performance. **DO NOT ACTIVATE THE STANDARD.**

1. To 125 µL sample add 25 µL **Sample Pretreatment Solution A**. Mix well.
2. Incubate 10 minutes at room temperature.
3. Add 25 µL of **Sample Pretreatment Solution B**. Mix well.
4. Add 800 µL of **Dilution Buffer**. Mix well and assay within 2 hours.

Note: 1) Sample results must be multiplied by the dilution factor, 7.8. If samples generate values higher than the highest standard, further dilute the samples after activation with Dilution Buffer and repeat the assay. 2) Do not activate the standard as it already contains active TGF-β3.
Use polypropylene tubes.

REAGENT PREPARATION

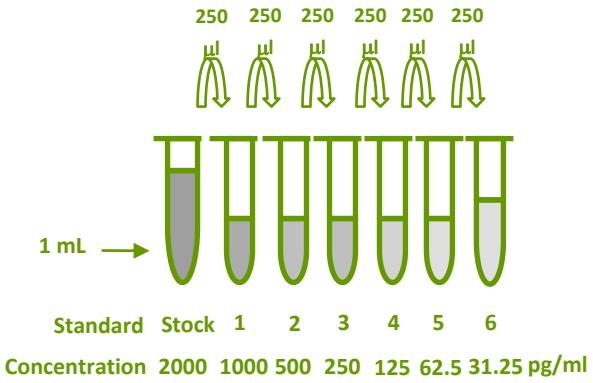
Bring all reagents to room temperature before use.

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 Wash Buffer.

TGF-β3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **TGF-β3** 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the

next transfer. The 2000 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 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



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

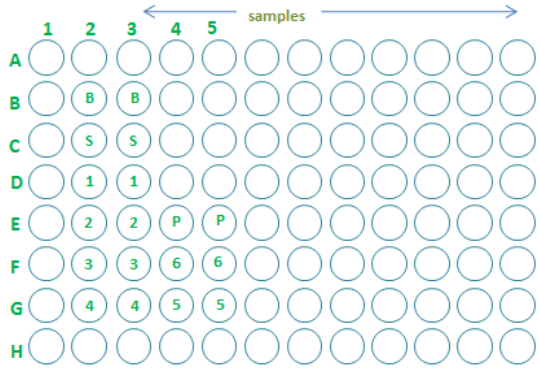
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

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 duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **Standard** (C2, C3 to G2, G3 and F4, F5 to G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 1 hour on micro-plate 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 7-13 minutes 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 micro-plate 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-β3 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 7.8 if samples were directly assayed after activation procedure. If samples required further dilution, then the concentration need to be multiplied by its dilution factor.

TYPICAL DATA

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

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.109)
31.25	0.089
62.5	0.157
125	0.279
250	0.536
500	1.008
1000	1.792
2000	2.682

- Lot:
- Positive Control: 115-220 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf*21-expressed recombinant TGF-β3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of TGF-β3 was 5 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human TGF-β3. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY (%)
Human TGF-β3	100
Human TGF-β1	0
Human TGF-β2	0
Human TGF-β4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of **standard, samples, positive control** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Streptavidin-HRP Conjugate working solution** to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Substrate Solution** to each well. Incubate 7-13 min on the plate shaker. **Protect from light**.
↓
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min