ab100647 – TGF beta 1 Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human TGF beta 1 in serum, plasma, and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 6 Last Updated 15 February 2016

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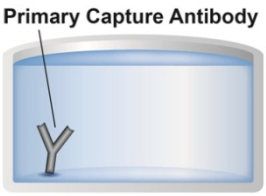
1. BACKGROUND

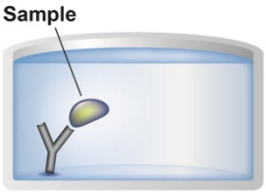
Abcam's TGF beta 1 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human TGF beta 1 in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for Human TGF beta 1 coated on a 96-well plate. Standards and samples are pipetted into the wells and TGF beta 1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human TGF beta 1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TGF beta 1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

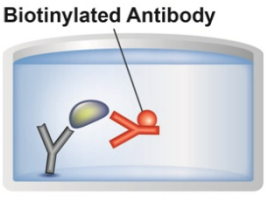
2. ASSAY SUMMARY



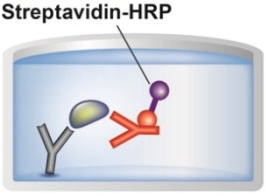
Prepare all reagents, samples and standards as instructed.



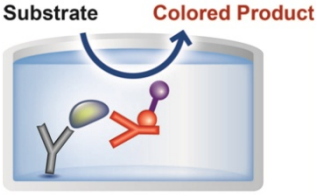
Add standard or sample to each well used. Incubate at room temperature



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
TGF beta 1 Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Assay Diluent A	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Biotinylated anti-Human TGF beta 1	2 vials	-20°C
Recombinant Human TGF beta 1 Standard	2 vials	-20°C
500X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Reagents required for Sample activation.

- **1 N HCl (100 ml) –** Slowly add 8.33 mL of 12 N HCl into 91.67 ml deionized water. Mix bottle.
- **1.2 N NaOH/0.5 M HEPES (100 ml) -** Slowly add 12 ml of 10 N NaOH into 75 mL deionized water. Mix bottle. Add 11.9 g HEPES. Mix through. Bring final volume to 100 mL with deionized water.
- **2.5 N Acetic Acid/10 M Urea (250 ml) -** Add 150.2 g of Urea into 100 mL deionized water. Mix bottle until dissolved. Slowly add 35.9 mL of Glacial Acetic Acid. Mix through. Bring final volume to 250 ml with deionized water.
- **2.7 N NaOH/1 M HEPES (250 ml) -** Add 67.5 ml of 10 N NaOH into 140 ml deionized water. Mix bottle. Add 59.5 g HEPES. Mix through. Bring final volume to 250 mL with deionized water.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 **1X Assay Diluent B**

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

9.2 **1X Wash Solution**

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Solution Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Solution.

9.3 **1X Biotinylated TGF beta 1 Detection Antibody**

Briefly spin the Biotinylated anti-Human TGF beta 1 vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 80-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

9.4 **1X HRP-Streptavidin Solution**

Briefly spin the 500X HRP-Streptavidin concentrate vial before use. HRP-Streptavidin concentrate must be diluted 500-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µL of 500X HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent B to prepare a final 500-fold diluted 1X HRP-Streptavidin solution. Mix well.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of TGF beta 1 Standard. Prepare a 300 ng/mL TGF beta 1 **Stock Standard** by adding 700 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into tube #1.

10.2 Dissolve the powder thoroughly by gentle mixing.

10.3 Label tubes #1-6.

10.4 Prepare **Standard #1** by adding 120 µL of the 300 ng/mL Stock Standard to 480 µL Assay Diluent A or 1X Assay Diluent B into tube 1#. Mix thoroughly and gently.

10.5 Pipette 400 µL Assay Diluent A or 1X Assay Diluent B into each tube.

10.6 Prepare **Standard #2** by transferring 200 µL from tube #1 to #2, mix thoroughly.

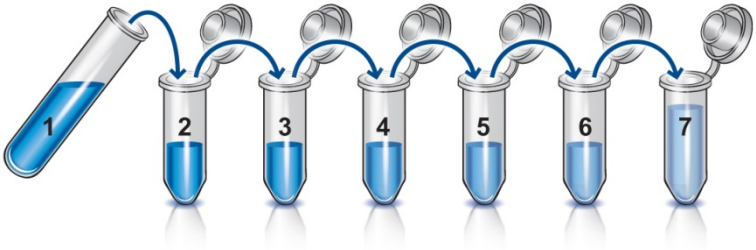
10.7 Prepare **Standard #3** by transferring 200 µL from tube #2 to #3, mix thoroughly.

10.1 Using the table below as a guide, prepare further serial dilutions.

10.2 Assay Diluent A or 1X Assay Diluent B serves as the zero standard, (0 ng/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	120	480	600	300	60
2	200	400	600	60	20
3	200	400	600	20	6.667
4	200	400	600	6.667	2.222
5	200	400	600	2.222	0.741
6	200	400	600	0.741	0.247
7	200	400	600	0.247	0.082
8	0	400	400	0	0



11. SAMPLE PREPARATION

General Sample Information:

- If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent B should be is used for dilution of culture supernatants.

TGF beta 1 Sample Activation Procedure:

- To activate latent TGF beta 1 to the immunoreactive form, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.0 – 7.6). Use polypropylene test tubes.
  Notes: Do not activate the kit standards. The kit standards contain active rhTGF beta 1.

Cell Culture Supernates

Add 0.1 mL 1 N HCL into 0.5 mL cell culture supernate. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutalize the acidified sample by adding 0.1 mL 1.2 N NaOH/0.5 M HEPES (pH 7.0 – 7.6). Mix tubes thoroughly. Assay immediately. The activated sample may be diluted with 1X Assay Diluent B. The concentration read off the standard curve must be multiplied by the dilution factor.

Serum/Plasma

Add 0.1 mL 2.5 N Acetic Acid/10 M Urea to 0.1 mL serum. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 mL 2.7 N NaOH/1 M HEPES. Mix tube thoroughly. Assay immediately. The activated sample may be diluted with Assay Diluent A. The concentration read off the standard curve must be multiplied by the dilution factor.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Add 100 µL of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3 Add 100 µL of 1X Biotinylated TGF beta 1 Detection Antibody (Reagent Preparation section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

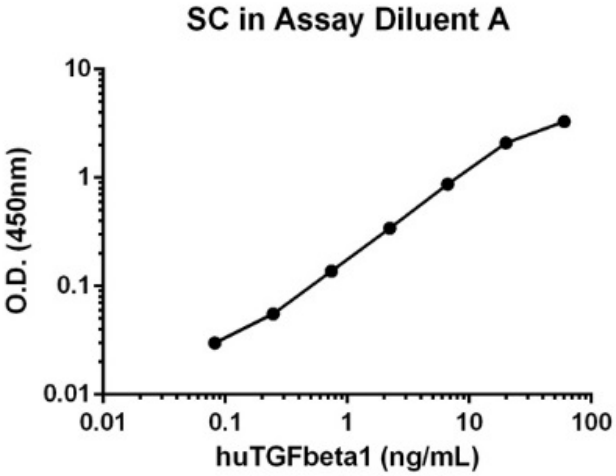
13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

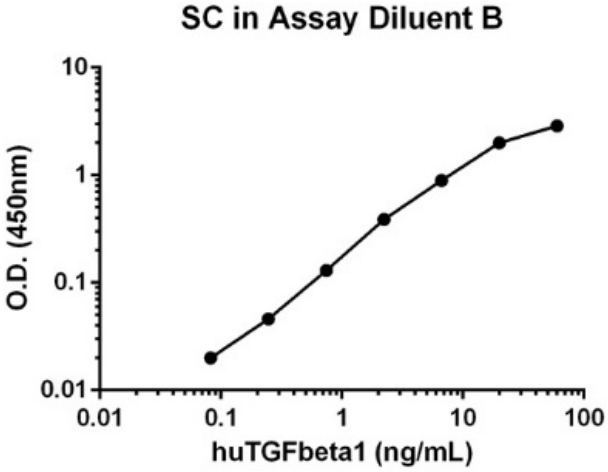
14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.





Conc. (ng/mL)	O.D.	
	Assay Diluent A	Assay Diluent B
0.082	0.03	0.020
0.247	0.0555	0.046
0.741	0.138	0.130
2.222	0.342	0.387
6.667	0.871	0.892
20	2.1	1.999
60	3.3	2.871

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of TGF beta 1 is typically less than 80 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of recombinant Human TGF beta 1 into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	94.46	82-102
Plasma	95.78	93-103
Cell Culture Media	97.87	85-104

LINEARITY OF DILUTION -

Serum Dilution	Average % Expected Value	Range (%)
1:2	92	82-103
1:4	93	83-105

Plasma Dilution	Average % Expected Value	Range (%)
1:2	95	83-104
1:4	94	84-105

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	95	84-104
1:4	94	83-104

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

17. ASSAY SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested; ANG, CD23, Eotaxin, GCSF, GM-CSF, GRO-α, GRO-β, GRO-γ, I-309, IFN-γ, IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-15, IL-16, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MCSF, MIG, MIP-1α, MIP-1β, NAP-2, PDGF, PF-4, PARC, SCF, SDF-1α, TIMP-1, TIMP-2, TNFβ, TGFβ2, TGFβ3, VEGF).

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measure.

19. NOTES

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