BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Total TGF-β1

Cat. No. 436707

ELISA Kit for Accurate Quantitation of Total TGF-β1 from Cell Culture Supernatant, Serum, Plasma and Other Biological Samples

BioLegend, Inc.

biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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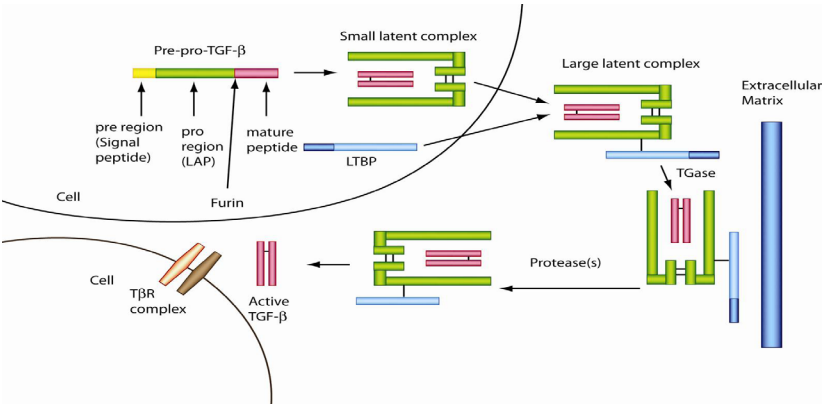
Introduction:

Transforming growth factor beta 1 (TGF-β1) is a member of the transforming growth factor beta superfamily of cytokines. The TGF-β1 precursor contains 390 amino acids with an N-terminal signal peptide of 29 amino acids required for secretion from a cell, a 249 amino acid pro-region (latency associated peptide or LAP), and a 112 amino acid C-terminal region that becomes the active TGF-β1 upon activation.

Both LAP and TGF-β1 exist as homodimers in circulation, but the disulfide linked homodimers of LAP and TGF-β1 remain non-covalently associated, forming the small latent TGFβ-1 complex (SLC, 100 kDa). The large latent TGF-β1 Complex (LLC, 235 – 260 kDa) contains a third component, the latent TGF-β binding protein (LTBP), which is linked to LAP by a single disulfide bond. The LTBP does not confer latency, but is for efficient secretion of the complex to extracellular sites. Free active TGF-β1 can be released (activated) by many factors including enzymes and low or high pH. The following picture illustrates the relationship among the different forms of TGF-β1.



TGF-β1 is nearly 100% conserved across mammalian species. It has diverse biological functions in multiple cellular processes such as regulating proliferation and differentiation of various cell types. TGF-β1 is also an important immunoregulatory cytokine, which is involved in the maintenance of self-tolerance, Th17 differentiation, and T-Cell homeostasis.

The BioLegend LEGEND MAX™ Total TGF-β1 ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-TGF-β1 capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-TGF-β1. This kit is specifically designed for the accurate quantitation of total TGF-β1 from cell culture supernatant, serum, plasma and other body fluids from many mammalian species (human, non-human primate, mouse, rat, porcine, bovine, ovine, rabbit, etc).

Tel: 858-768-5800

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-TGF-β1 Pre-coated 96-well Microplate	1 plate		78242
TGF-β1 Detection Antibody	1 bottle	12 mL	78243
TGF-β1 Standard	1 vial	lyophilized	78251
Avidin-HRP D	1 bottle	12 mL	78237
Assay Buffer C	1 bottle	25 mL	78247
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Sample Diluent	1 bottle	25 mL	78248
Acidification Solution	1 vial	1.5 mL	78260
Neutralization Solution	1 vial	1.5 mL	78250
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="10">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP D</td></tr>
<tr><td>Assay Buffer C</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Acidification Solution</td></tr>
<tr><td>Neutralization Solution</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution and Acidification Solution contain a strong acid. Neutralization Solution contains a strong base. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Tel: 858-768-5800

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

Note: All reagents should be diluted immediately prior to use. Do not use sodium azide in any solutions as it inhibits the activity of the horseradish peroxidase enzyme, greatly reducing the signal in each well.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized TGF-β1 Standard by adding the volume of Assay Buffer C to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

Sample Preparation:

Note: For measuring total TGF-β1, samples require treatment to activate TGF-β1.

Serum/Plasma Samples: Add 10 µL of serum/plasma to a polypropylene microfuge tube, then add 5 µL of Acidification Solution, mix well, and incubate for 10 minutes at room temperature. Add 5 µL of Neutralization Solution, mix well.

Dilute treated serum/plasma samples in Sample Diluent according to estimated sample concentration, which can be determined by a pilot study. For example, to make a 50 fold dilution of treated serum/plasma samples in a final volume of 250 µL, dilute 5 µL treated serum/plasma samples in 245 µL Sample Diluent (final dilution factor is 100).

Cell Culture Supernatant Samples: Add 40 µL of cell culture supernatant sample to a polypropylene microfuge tube, then add 10 µL of Acidification Solution, mix well, and incubate for 10 minutes at room temperature. Add 10 µL of Neutralization Solution, mix well.

Dilute treated cell culture supernatant in Sample Diluent according to estimated sample concentration, which can be determined by a pilot study.

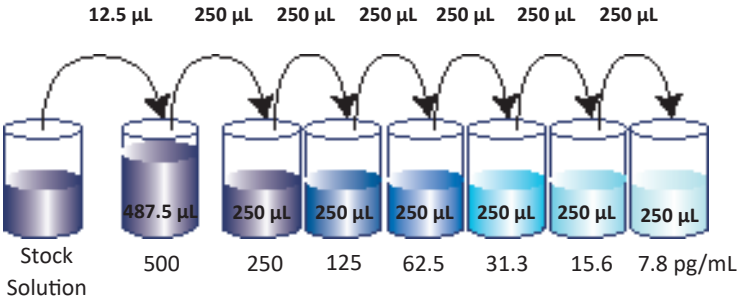
For example, to make a 2 fold dilution of treated serum free cell culture supernatant samples in Sample Diluent, dilute 60 µL treated serum free cell culture supernatant samples in 60 µL Sample Diluent (final dilution factor is 3).

If cells have been cultured in media containing serum (*e. g.* 10% fetal bovine serum), the media should be treated using the same protocol as a control because the significant levels of TGF-β1 are present in the animal serum. To measure TGF-β1 in serum-containing cell culture supernatants, a large dilution of treated samples is required prior to the assay. For example, to make a 50 fold dilution of treated samples in a final volume of 250 µL, dilute 5 µL treated samples in 245 µL Sample Diluent (final dilution factor is 75).

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

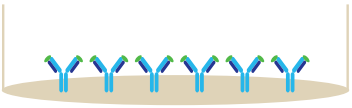
1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500 µL of the 500 pg/mL top standard by diluting 12.5 µL of the standard stock solution in 487.5 µL Assay Buffer C. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes using Assay Buffer C as the diluent. Thus, the TGF-β1 Standard concentrations in the tubes are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. Assay Buffer C serves as the zero standard (0 pg/mL).



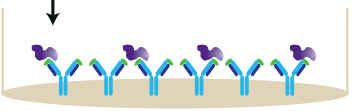
4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 µL of Assay Buffer C to each well that will contain either standard dilutions or samples.
6. Add 50 µL of standard dilutions or samples to the appropriate wells.
7. Seal the plate with the plate sealer included in the kit and incubate the plate at room temperature for 2 hours with shaking at 200 rpm.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 µL of TGF-β1 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP D solution to each well, seal the plate and incubate at room temperature for 30 minutes with shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing total TGF-β1 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

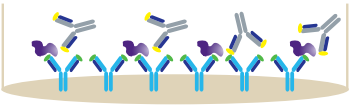
1. Wash 4 times
Add 50 µL Assay Buffer C



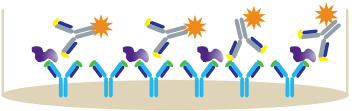
2. Add 50 µL diluted standards or samples
Incubate 2 hrs, RT, shaking



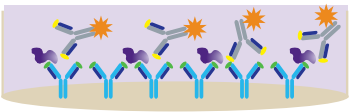
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



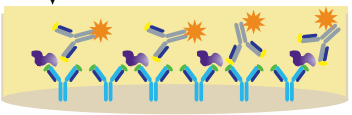
4. Wash 4 times
Add 100 µL Avidin-HRP D solution
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 10 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

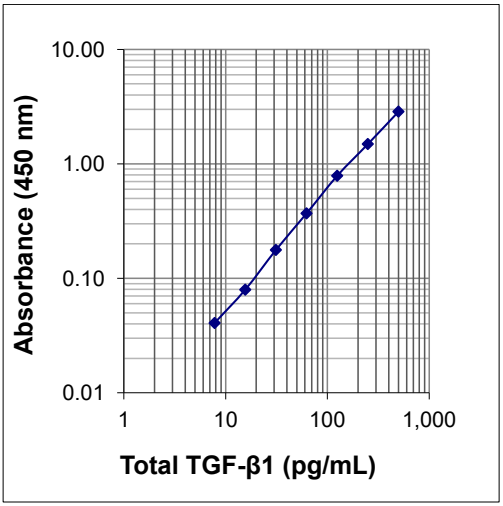
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

Because serum/plasma and cell culture supernatant samples have been diluted prior to the assay, their measured concentrations must be multiplied by the dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross reactivity was observed when this kit was used to analyze recombinant latency-associate peptide (TGF-β1) at up to 40 ng/mL. No cross reactivity was observed when this kit was used to analyze 69 human, 43 mouse and 35 rat cytokines/chemokines at up to 50 ng/mL.

Sensitivity: The minimum detectable concentration of TGF-β1 is 3.5 pg/mL.

Recovery: Recombinant TGF-β1 (500, 250, 125, 62.5, 31.3, 15.6, and 7.8 pg/mL) was spiked into 5 treated human serum samples, and then analyzed with the LEGEND MAX™ Total TGF-β1 ELISA kit. On average, 74% of the cytokine was recovered from the serum samples.

Linearity: After treatment, 4 human serum, 2 mouse serum, and 1 rabbit serum samples were diluted 1:50, 1:100, 1:200, with Sample Diluent to produce samples with values wthin the dynamic range. On average, 98% of the expected cytokine was detected from the serum samples.

Intra-Assay Precision: Two samples containing different TGF-β1 concentrations were tested in one plate with 16 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	26.5	113.9
Standard Deviation	0.9	4.4
% CV	3.4	3.9

Inter-Assay Precision: Two samples containing different concentrations of TGF-β1 were tested in seven independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	7	7
Mean Concentration (pg/mL)	27.7	121.6
Standard Deviation	1.8	8.0
% CV	6.5	6.6

Normal Sample Values:

Serum	Human (ng/mL)	Mouse (ng/mL)	Rat (ng/mL)	Porcine (ng/mL)
Number of Samples	14	4	4	1
Median	3.9	75.5	50.9	>25.0
Maximum	41.3	105.6	57.7	
Minimum	1.7	59.4	25.8	

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

ELISA Plate Template	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
8999 BioLegend Way
San Diego, CA 92121
Tel: 1.858.768.5800
Tel US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com

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