

Human LAP(TGF-Beta1) ELISA Kit

Cat #: orb178693 (manual)

For the quantitation of Human LAP(TGF-Beta1) concentrations in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

Assay Principle

The Human LAP(TGF-Beta1) ELISA Kit is a solid-phase immunoassay specially designed to measure Human LAP(TGF-Beta1) with a 96-well strip plate that is pre-coated with antibody specific for LAP(TGF-Beta1). The detection antibody is a biotinylated antibody specific for LAP(TGF-Beta1). The kit includes Human LAP(TGF-Beta1) protein as standards.

To measure Human LAP(TGF-Beta1), add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbound ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human LAP(TGF-Beta1) in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human LAP(TGF-Beta1) in the sample.

Overview

Product Name	Human LAP(TGF-Beta1) ELISA Kit
Reactive Species	Human
Size	96 wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human LAP (TGF- beta1). 96wells/kit, with removable strips.
Sensitivity*	<10 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	62.5 pg/ml - 4,000 pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.
Uniprot ID	P01137

Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human LAP(TGF-Beta1)
Immunogen	Expression system for standard: sf21; Immunogen sequence: L30-R278
Cross-reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human LAP(TGF-Beta1) Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells
Human LAP(TGF-Beta1) Standard	2	10 ng/tube
Human LAP(TGF-Beta1) Biotinylated Antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30 ml
Antibody Diluent	1	12 ml
Avidin-Biotin-Peroxidase Diluent	1	12 ml
Color Developing Reagent (TMB)	1	10 ml
Stop Solution	1	10 ml
Wash Buffer (25x)	1	20 ml
Plate Sealers	4	piece

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450 nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water. 500ml graduated cylinders.

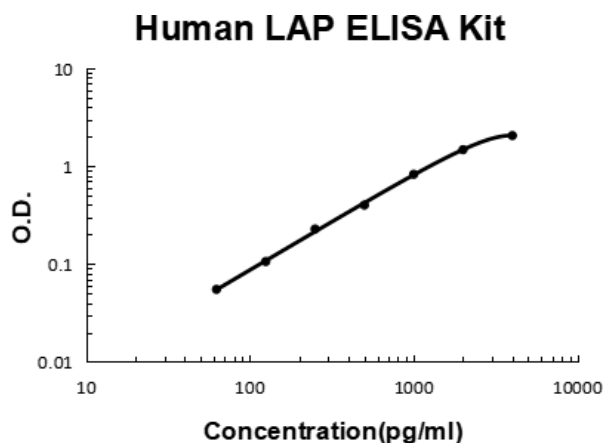
Test tubes for dilution.

Human LAP(TGF-Beta1) ELISA Standard Curve Example

The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration(pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.004	0.059	0.110	0.232	0.404	0.831	1.479	2.261

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



Intra/Inter-Assay Variability

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24

Mean (pg/ml)	87	673	2000	90	698	2085
Standard deviation	3.91	43.74	126	4.5	57.93	148.35
CV (%)	4.5%	6.5%	8.3%	5%	8.3%	7%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot 1 (pg/ml)	Lot 2 (pg/ml)	Lot 3 (pg/ml)	Lot 4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	87	104	88	101	95	7.58	7.9%
Sample 2	673	684	619	721	674	36.51	5.4%
Sample 3	2000	1784	2011	1799	1898	107.2	5.6%

*number of samples for each test n=16.

Preparation Before the Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37 °C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human LAP(TGF-Beta1) antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human LAP(TGF-Beta1) Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human LAP(TGF-Beta1) Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human LAP(TGF-Beta1) standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Human LAP(TGF-Beta1) Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –4000pg/ml, #2 –2000pg/ml, #3 – 1000pg/ml, #4 – 500pg/ml, #5 – 250pg/ml, #6 – 125pg/ml, #7 – 62.5pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).
2. To generate standard #1, add 400µl of the reconstituted standard stock solution of 10ng/ml and 600µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately, or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma, for other anticoagulants could block the antibody binding site.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant.

*Note: To detect LAP(TGF-Beta1) in samples, you need to activate LAP(TGF-Beta1) in samples prior to the assay.

There is no detectable cross-reactivity with other relevant proteins LAP (TGF-beta1) is mostly contained as inactive form in samples, please activate it before assay. Don't activate recombinant LAP (TGF-beta1).
Solution A: 1N HCl: add 8.33ml of 12N HCl into 91.67ml of H₂O. Solution B: 1.2N NaOH/0.5M HEPES: add 12ml of 10N NaOH and 11.9g HEPES into 75ml of H₂O, add H₂O to adjust volume to 100ml.

Cell culture supernates: add activating reagent pro rata, i.e. add 20ul of Solution A into 100ul of sample, 10 min later, add 20ul of Solution B. PH 7.0-7.6. Serum, plasma (heparin, EDTA or citrate): add activating reagent pro rata, i.e. add 20ul of Solution A into 40ul of sample, 10 min later, add 20ul of Solution B. PH 7.0-7.6. It is unnecessary to activate the recombinant LAP (TGF-beta1). Sample was diluted partly after adding activating reagent, so please pay attention to this when calculate target protein concentration.

Sample Collection Notes

The target protein concentration should be estimated, and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Protocol

It is recommended that all reagents and materials be equilibrated to 37 °C/ room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human TGF-BETA1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.

13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on LAP(TGF-Beta1)

LAP(TGF-Beta1) is secreted as a latent form, which consists of its mature form and a latency-associated peptide (beta1-LAP) in either the presence or the absence of additional latent TGF-beta1-binding protein. Processing and cleavage of the precursor protein between amino acids 278 and 279 results in the formation of LAP dimers and TGF beta dimers that then non-covalently associate with each other to form the small latent TGF beta complex. LAP(TGF-Beta1) is secreted and can be found in the extracellular matrix. In addition, LAP(TGF-Beta1) can also be expressed on platelets and activated regulatory T cells.