Human Latent TGF-β1 ELISA Kit

For detection and measurement of human latent transforming growth factor-beta 1

Catalog #02018 2 Plates
Catalog #02019 10 Plates

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Product Description

The Human Latent Transforming Growth Factor-beta 1 (TGF-β1) Kit is designed for the quantitative detection and measurement of latent human TGF-β1 in biological fluids such as plasma and cell culture supernatants. TGF-β1 is produced primarily by platelets and bone, and in smaller amounts by most nucleated cells and tumors. TGF-β1 stimulates cells of mesenchymal origin, but inhibits cells of epithelial or neuroectodermal origin. TGF-β1 associates with latency associated protein (LAP) to form a latent complex. This kit detects the LAP entity of the complex.

The assay is based on the sandwich ELISA method, in which samples are added to ELISA strip plates pre-coated with capture antibodies specific for the cytokine. The captured cytokine is detected by addition of a biotinylated detection antibody, followed by streptavidin-horseradish peroxidase, which binds the biotinylated antibody. Addition of the chromogenic enzyme substrate 3,3',5,5' tetramethyl-benzidine (TMB) results in a colored product with an intensity directly proportional to the concentration of cytokine in the sample. The concentration of the cytokine is determined by comparison to serial dilutions of the cytokine standard analyzed in parallel.

Product Information

All components listed below are stable until expiry date (EXP) on label. Once components are opened, use within 1 month. Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated upon receipt.

COMPONENT NAME	2-PLATE KIT		10-PLATE KIT		STORAGE	DESCRIPTION
	COMPONENT #	SIZE	COMPONENT #	SIZE		
Human TGF-β1 LAP Standard	02018B	1 vial	02018B	1 vial	Store at -20°C.	Recombinant human latency associated protein (LAP)
Human Latent TGF-β1 ELISA Plate	02018C	2 plates	02018C	10 plates	Store at 2 - 8°C.	Plate (12 strips x 8 wells) coated with anti-human latent TGF-β1 antibody
Human Latent TGF-β1 Biotinylated Detection Antibody	02018D	50 µL	02019D	250 µL	Store at 2 - 8°C.	Biotinylated anti-human latent TGF-β1 antibody (0.5 mg/mL)
Adhesive Plate Covers	01901	6 covers	01902	30 covers	Store at 2 - 8°C.	For covering plates during incubation
Stop Solution*	01903	25 mL	01904	120 mL	Store at 2 - 8°C.	0.18 M H_2SO_4
TMB Substrate	01905	25 mL	01906	120 mL	Store at 2 - 8°C.	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide
SA-HRP Diluent	01907	25 mL	01908	120 mL	Store at 2 - 8°C.	For dilution of SA-HRP
ELISA Diluent	01909	120 mL	01909	3 x 120 mL	Store at 2 - 8°C.	Protein-containing buffer for dilution of samples, standard, and detection antibody
Wash Buffer (20X)	01910	120 mL	01910	5 x 120 mL	Store at 2 - 8°C.	Concentrated buffer solution for washing plates between steps
Standard Reconstitution Buffer C	01913	1 mL	01913	1 mL	Store at 2 - 8°C.	For reconstitution of lyophilized cytokine standard
SA-HRP	01914	30 µL	01915	130 µL	Store at 2 - 8°C.	Streptavidin-horseradish peroxidase conjugate

*Please refer to the Safety Data Sheet (SDS) for hazard information.

Materials Required But Not Included

- Biohazard safety cabinet certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm and (optional) a second correction wavelength of 650 nm
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Micropipette (e.g. Eppendorf, Gilson) with appropriate tips
- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for standard and sample dilutions
- Timer
- Absorbent paper
- Deionized water

Preparation of Reagents and Materials

A. Wash Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

Dilute Wash Buffer (20X) 1 in 20 with distilled or deionized water.

Example: For one plate, prepare 1000 mL wash buffer by adding 50 mL Wash Buffer (20X) to 950 mL distilled or deionized water.

B. ELISA Plates

1. Allow plates to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include a standard curve and a background control (6 x 2 wells), a blank (2 wells), and sample wells.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the foil bag containing the desiccant at 2 - 8°C.
4. Wash the strips with 5 x 300 µL/well of wash buffer (prepared in section A). Ensure wash buffer is thoroughly removed from the wells by firmly tapping the plate upside down on absorbent paper.

C. Standard Stock Solution

1. Add 1 mL of Standard Reconstitution Buffer C to the vial of Human TGF-β1 LAP Standard (final concentration 20 nM). Let sit at room temperature (15 - 25°C) for 5 minutes.
2. Vortex the tube, centrifuge briefly, and aliquot.

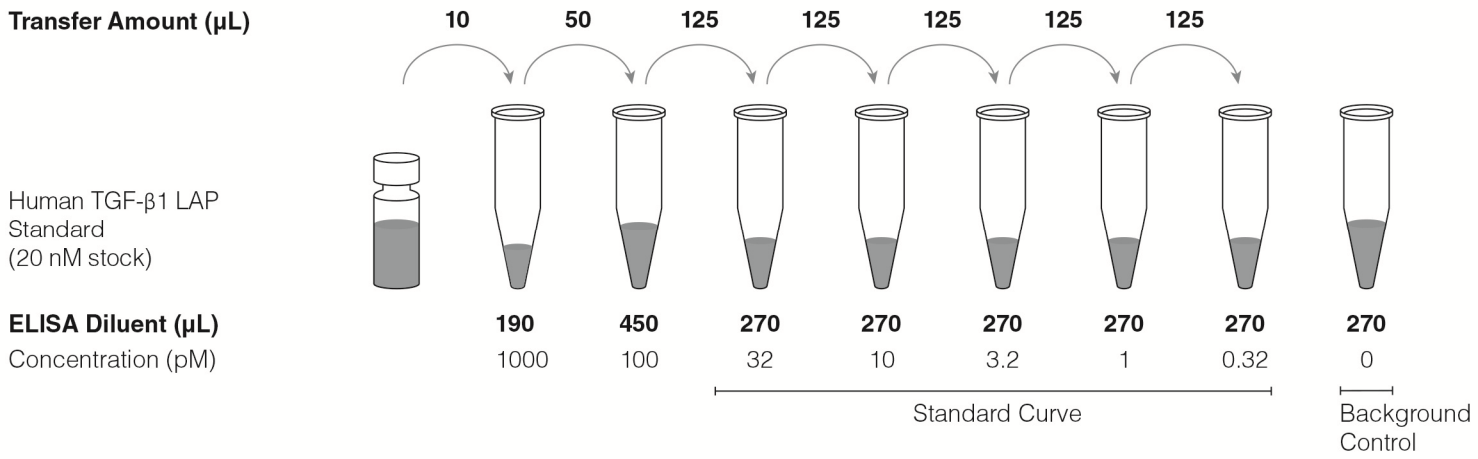
   NOTE: If not used immediately, store aliquots at -20°C for up to 1 month. After thawing the aliquots, do not re-freeze.

D. Standard Curve

NOTE: The standard curve dilutions may be prepared from freshly prepared standard stock solution or from thawed aliquots. Prepare the standard curve dilutions no more than 30 minutes prior to beginning the assay. Duplicate wells for the standard curve dilutions and the background control are recommended.

Dilute the standard stock solution (prepared in section C) to create standard curve dilutions ranging from 0.32 - 32 pM according to Figure 1.

For the background control (0 pg/mL) use only ELISA Diluent.



NOTE: Volumes indicated are sufficient for duplicates.

Figure 1. Recommended Serial Dilution of Cytokine Standard

E. Samples

NOTE: Avoid using lipemic, hemolysed, or contaminated samples as these may yield unreliable results.

To quantify latent TGF-β1 in blood, the use of plasma in this ELISA is recommended. Plasma can be obtained using EDTA, citrate, or heparin as anticoagulants. To minimize platelet content, centrifuge plasma at 10,000 x *g* for 10 minutes.

Dilute all samples at least 1:1 in ELISA Diluent in tubes, for a total minimum volume of 250 µL per dilution. Samples containing high levels of cytokine (exceeding the range of the standard curve) will require further dilution. Mix thoroughly. Pre-treatment of samples is not required for dissociation of the latent TGF-β1 complex.

NOTE: Duplicate wells for all samples are recommended. For samples with a high cytokine concentration, it is advisable to prepare several dilutions for testing. Remove any particulate matter by centrifugation or filtration prior to use.

F. Detection Antibody

Dilute the Human Latent TGF-β1 Biotinylated Detection Antibody 1 in 500 in ELISA Diluent.

Example: For one plate, add 24 µL of Human Latent TGF-β1 Biotinylated Detection Antibody to 12 mL of ELISA Diluent (final concentration 1 µg/mL).

G. SA-HRP

Dilute SA-HRP 1 in 1000 in SA-HRP Diluent.

Example: For one plate, add 12 µL SA-HRP to 12 mL SA-HRP Diluent.

Directions for Use

Please read the entire protocol before proceeding.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

NOTE: In all washing steps, each well must be thoroughly washed and blotted dry by tapping the plate upside down on absorbent paper.

1. Bring all reagents and samples to room temperature (15 - 25°C), except the TMB Substrate, which should be kept at 2 - 8°C until use.
2. Prepare all reagents, samples, standard dilutions, and ELISA plates as described in Preparation of Reagents and Materials.
3. Add 100 µL/well of each standard curve dilution and background control (see Preparation of Reagents and Materials, section D). Leave the blank wells empty.
4. Add 100 µL/well of diluted sample (see Preparation of Reagents and Materials, section E).

   NOTE: Duplicate wells for all samples are recommended.
5. Cover the plate with an Adhesive Plate Cover and incubate at room temperature (15 - 25°C) for 2 hours.
6. Wash each well with 5 x 300 µL of wash buffer (see Preparation of Reagents and Materials, section A) and blot dry.
7. Add 100 µL/well of diluted detection antibody (see Preparation of Reagents and Materials, section F). Leave blank wells empty.
8. Cover the plate with an Adhesive Plate Cover and incubate at room temperature (15 - 25°C) for 1 hour.
9. Wash each well with 5 x 300 µL of wash buffer and blot dry.
10. Add 100 µL/well of diluted SA-HRP (See Preparation of Reagents and Materials, section G). Leave blank wells empty.
11. Cover the plate with an Adhesive Plate Cover and incubate at room temperature (15 - 25°C) for 1 hour.
12. Wash each well with 5 x 300 µL of wash buffer and blot dry.
13. Add 100 µL/well of TMB Substrate to all wells (including blank wells).
14. Incubate at room temperature (15 - 25°C) in the dark for 15 minutes.
15. Add 100 µL/well of Stop Solution to all wells (including blank wells).

    NOTE: Use care when handling Stop Solution. Please refer to the Safety Data Sheet (SDS) for hazard information.
16. Within 15 minutes of adding Stop Solution, measure the absorbance at 450 nm in a microplate reader. If possible, use a correction wavelength of 650 nm. Blank the reader using ELISA wells containing TMB Substrate and Stop Solution only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
17. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the standard, the background control, and the sample values prior to generating the standard curve and determining the cytokine concentrations in the samples. A representative standard curve is shown in Figure 2.

    NOTE: Multiply the cytokine concentrations by the dilution factor used for each sample.

Performance of the Assay

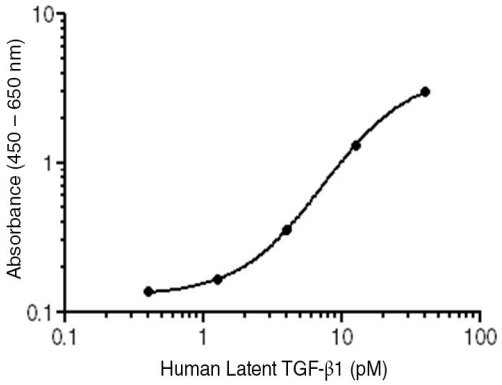


Figure 2. Representative Standard Curve

- **Reportable range**: 0.32 - 32 pM. This is the concentration range in which measurement of the analyte can be done with the highest precision, accuracy, and linearity.

  Recombinant human LAP (latency associated protein) homodimer is the analyte standard of this ELISA. Determination of latent TGF-β1 using the standard curve is based on a molar comparison. 1 pM LAP (54 pg/mL) corresponds to 1 pM latent TGF-β1 (80 pg/mL).
- **Sensitivity**: The limit of detection of this assay is 0.13 pM. This is the analyte concentration with absorbance two standard deviations higher than the zero standard.
- **Recovery**: A mid-curve recovery of 100% was determined by dilution of plasma samples in repeated experiments.
- **Precision**: The intra-assay precision of this assay is 3.0% (CV). The inter-assay precision of this assay is 10.4% (CV).

Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Absorbance values too low	Incubation time too short	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Undiluted Wash Buffer (20X)	Dilute Wash Buffer (20X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A.
	Incorrect dilution of Detection Antibody and/or SA-HRP	Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials.
	Wash step omitted before adding SA-HRP	Follow sequence of incubation and wash steps as outlined in Directions for Use.
Absorbance values too high	Incubation time too long	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Temperature too high (> 25°C)	Perform assay at a lower temperature. If that is not possible, reduce incubation times.
	Incorrect dilution of Detection Antibody and/or SA-HRP	Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials.
Low absorbance readings despite good color development in wells	Incorrect wavelength setting on the ELISA reader	Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength.
High background	Insufficient washing	Ensure that each well is washed 5X with 300 µL of diluted Wash Buffer (20X). Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Incorrect dilution of Detection Antibody and/or SA-HRP	Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials.
No signal with standard curve dilutions	Reagents not added in correct sequence	Follow sequence of incubation and wash steps as outlined in Directions for Use.
	Incorrect dilution of Detection Antibody and/or SA-HRP	Follow the directions for dilution of Detection Antibody and SA-HRP in Preparation of Reagents and Materials.

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Inconsistent dose-response curve	Incubation conditions for individual wells not identical	Avoid delays during filling of assay wells; ensure that all reagents are at 15 - 25°C prior to beginning the assay.
Poor replicates	Insufficient washing	Ensure that each well is washed 5X with 300 µL of diluted wash buffer. Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Unequal volumes in wells	Ensure that pipettes function properly.
	Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover	Always use a new adhesive cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution.
	Samples not mixed after thawing	Vortex samples after thawing.
	High lipids or particulate matter in samples	Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed.
	Air bubbles in well during measurement with the ELISA reader	Remove air bubbles using a small hypodermic needle or pipette tip.
Absorbance values of sample dilutions decrease with increasing concentration	Cytokine concentration of the sample dilutions exceeds the upper limit of the assay	Dilute the samples further and retest along with the standard curve dilutions.

Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit our website at www.stemcell.com or contact us at techsupport@stemcell.com.