ELISA for Human Latent TGF-β1

Product Code: 3550-1H-20

CONTENTS, development kit for 20 plates:

Vial 1 (green top)
Monoclonal antibody MT593 (1000 µl)
Concentration: 0.5 mg/ml

Vial 2 (red top)
Biotinylated monoclonal antibody MT517 (500 µl)
Concentration: 0.5 mg/ml

Vial 3 (white top)
Streptavidin-Horseradish Peroxidase (250 µl)

Vial 4
Recombinant human LAP standard

To ensure total recovery of stated quantity, vials have been overfilled.

STORAGE:
Shipped at ambient temperature. On arrival box 1 should be stored refrigerated at 4-8°C and box 2 should be stored frozen at -20°C.

General

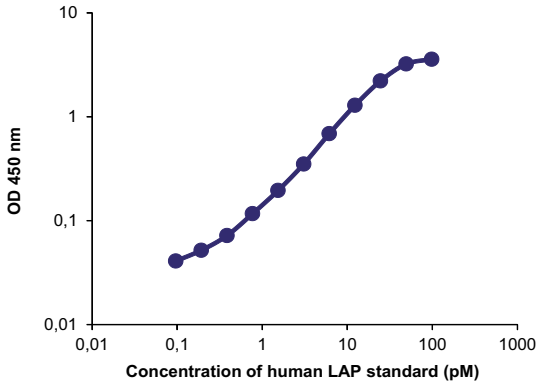
Intended use: For quantitative determination of native and recombinant human latent Transforming Growth Factor-β1(TGF-β1) in solution, e.g. cell culture supernatant. Analysis of latent TGF-β1 by this ELISA does not require any pre-treatment of samples to dissociate the latent complex. The monoclonal antibodies MT593 and MT517-biotin bind to the Latency Associated Protein (LAP), which is a part of the latent TGF-β1 complex. The ELISA does not detect human latent TGF-β2 or -β3 or bovine latent TGF-β1.

Serum/plasma samples: For quantification of latent TGF-β1 in blood, the use of plasma is recommended since serum contains high levels of latent TGF-β1 released from platelets during sample preparation. Plasma can be obtained using EDTA, citrate or heparin as anti-coagulants. To minimize the platelet content in the sample, an additional centrifugation of plasma at 10,000 x g for 10 minutes is recommended. Please note that cytokine determinations in plasma require the use of ELISA diluent (product code: 3652-D2) for dilution of samples, standard and detection antibody. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies found in plasma and serum. The ELISA diluent has been validated using plasma from normal healthy human blood donors. Heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed.

Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.15% Kathon CG.

Standard range: 0.5-50 pM

Standard calibration: Recombinant human LAP (homodimer) is used as standard. Since it differs in molecular weight from latent TGF-β1, determination of latent TGF-β1 using the standard curve is based on a molar comparison. A concentration of 1 pM LAP corresponds to 1 pM latent TGF-β1. For conversion from pM to pg/ml: 1 pM LAP = 54 pg/ml and 1 pM latent TGF-β1 = 80 pg/ml.



Guidelines for Human Latent TGF-β1 ELISA

Day 1 1. Coat a high protein binding ELISA plate with mAb MT593, diluted to 2 µg/ml in PBS, pH 7.4, by adding 100 µl/well. Incubate overnight at 4-8°C.

Day 2 2. Wash twice with PBS (200 µl/well).

3. Block plate by adding 200 µl/well of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.

4. Wash five times with PBS containing 0.05% Tween20.

5. Prepare LAP standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA to make up a stock solution of 120 nM. Leave at room temperature for 15 minutes and then vortex the tube. The stock solution should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not be refrozen after initial use. For the test, prepare dilutions of the stock using the standard range as a guideline.

6. Add 100 µl/well of samples or standards diluted in incubation buffer or ELISA diluent for plasma samples and incubate for 2 hours at room temperature.

7. Wash as in step 4.

8. Add 100 µl/well of mAb MT517-biotin at 1 µg/ml in incubation buffer or ELISA diluent for plasma samples. Incubate for 1 hour at room temperature.

9. Wash as in step 4.

10. Add 100 µl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. **Please note that sodium azide used in buffers will inhibit HRP activity.**

11. Wash as in step 4.

12. Add 100 µl/well of appropriate substrate solution.

13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

NOTE; for research use only.
MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages therefrom.

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