MABTECH

ELISA Flex: Human Latent TGF-β1 (HRP)

3550-1H-6 | 3550-1H-20

ELISA Flex kit for quantitative determination of native and recombinant human latent Transforming Growth Factor-β1 (TGF-β1) in solution, e.g. cell supernatant.

The kit includes		3550-1H-6 for 6 plates	3550-1H-20 for 20 plates
Capture mAb:	MT593 (0.5 mg/ml)	300 µl	1000 µl
Detection mAb:	MT517, biotin (0.5 mg/ml)	150 µl	500 µl
Streptavidin-HRP		80 µl	250 µl
Recombinant human LAP (homodimer) ELISA standard		1 vial	1 vial
Standard reconstitution buffer A8		1 ml	1 ml

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

Shipping and storage

Shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard which should be stored at -20 °C. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

General and Preparations

Specificity

The kit contains a matched pair of monoclonal antibodies (mAbs) specific for LAP entity of human latent TGF-β1. This enables direct quantification of latent TGF-β1 in ELISA with no need of sample pre-treatment. The ELISA does not detect human latent TGF-β2 or -β3 or bovine latent TGF-β1. The mAbs in this kit cross-react with latent TGF-β1 from non-human primates. Please visit www.mabtech.com for reactivity on NHP species.

Standard range

0.5-50 pM

Calibration

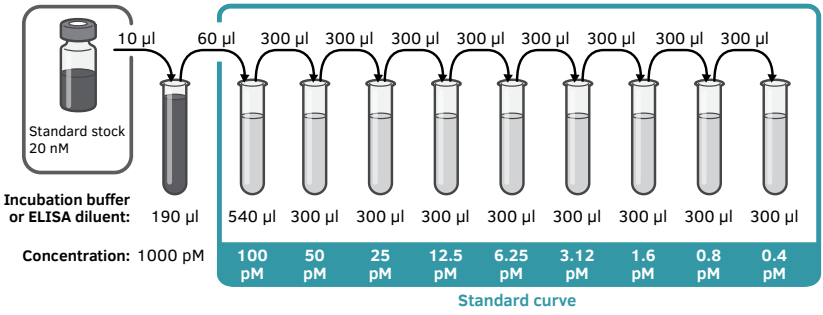
Since the included LAP standard differs in molecular weight from latent TGF-β1, determination of latent TGF-β1 using the standard curve is based on a molar comparison. 1 pM LAP corresponds to 1 pM latent TGF-β1. For conversion from pM to pg/ml: 1 pM LAP = 54 pg/ml and 1 pM latent TGF-β1 = 80 pg/ml.

Analysis of serum and plasma samples

Minimize platelet content in plasma by an additional centrifugation at 10,000 x g for 10 min in connection with the plasma preparation. Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of standard, samples, and detection antibody.

Reconstitution of ELISA standard

Reconstitute the standard to a stock solution of 20 nM by adding 1 ml of the standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles. Prepare the standard curve within 30 minutes of use.



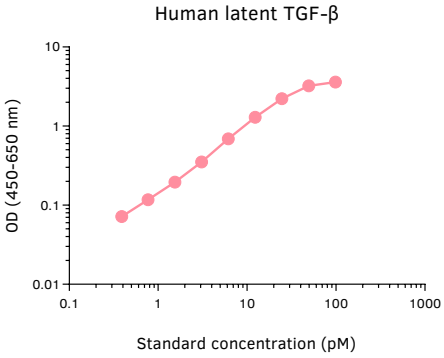
Protocol

Day 1

1. Add 100 µl/well of capture mAb MT593 diluted to 2 µg/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °C.

Day 2

2. Empty the plate and add 200 µl/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 µl/well).
4. Add 100 µl/well of samples or standards diluted in incubation buffer or ELISA diluent. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100 µl/well of detection mAb MT517-biotin diluted to 1 µg/ml in incubation buffer or ELISA diluent. Incubate for 1 hour at room temperature.
7. Wash as above.
8. 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.
9. Wash as above.
10. Add 100 µl/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
11. Add 100 µl/well of 0.2 M H_2SO_4 to stop the reaction.
12. Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



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