MABTECH

ELISA Flex: Human Thioredoxin-1 (HRP)

3580-1H-6 | 3580-1H-20

ELISA Flex kit for quantitative determination of native and recombinant, reduced and oxidized forms of human Thioredoxin-1 (Trx1) in solution, e.g. cell supernatant and serum/plasma.

The kit includes		3580-1H-6 for 6 plates	3580-1H-20 for 20 plates
Capture mAb:	MT17R6 (0.5 mg/ml)	300 µl	1000 µl
Detection mAb:	MT13X3, biotin (5 µg/ml)	150 µl	500 µl
Streptavidin-HRP		80 µl	250 µl
Recombinant human Thioredoxin-1 ELISA standard		1 vial	1 vial
Standard reconstitution buffer A5		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. MAbs are supplied in sterile PBS with sodium azide (0.02%). MAb MT13X3 contains 0.5% BSA. 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 native and recombinant human Trx-1. The ELISA detects reduced and oxidized forms of human Trx1. The mAb cross-react with bovine Trx1, thus cell supernatants must be free of bovine serum.

Standard range

20-2000 pg/ml

Calibration

No international standard exists for calibration

Analysis of serum and plasma samples

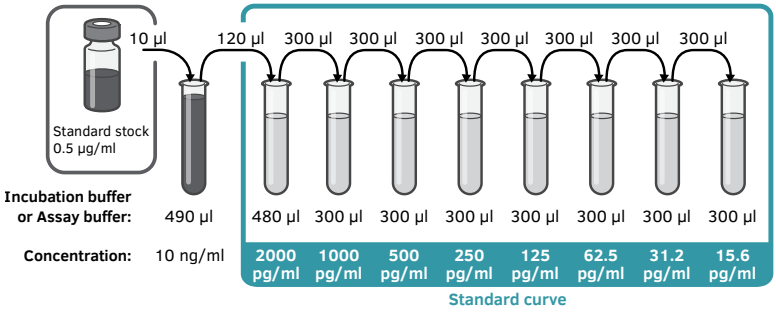
Analysis of Trx-1 in plasma is recommended since serum contains high levels of Trx1 released from platelets. Minimize platelet content in plasma by an additional centrifugation at 10,000 x g for 10 min in connection with the plasma preparation. Analysis requires the use of Assay buffer (product code: 3652-J2). The buffer blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The Assay buffer should be used for dilution of standard, samples, and detection antibody.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.5 µg/ml by adding 1 ml of the standard reconstitution buffer. Allow the standard to dissolve for 15 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of standard curve

Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



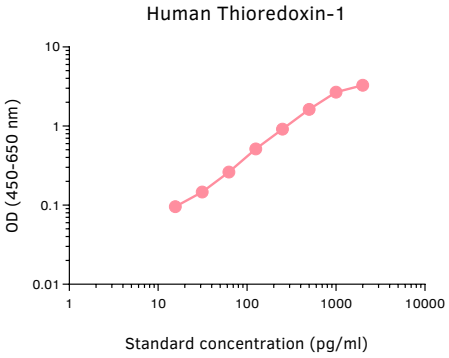
Protocol

Day 1

1. Add 100 µl/well of capture mAb MT17R6 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 Assay buffer. 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 MT13X3-biotin diluted to 0.01 µg/ml in incubation buffer or Assay buffer. 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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