

# Human Thioredoxin-1 ELISA development kit

Product Code: 3580-1A-6

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CONTENTS, development kit for 6 plates:

**Vial 1 (green top)**

Monoclonal antibody MT17R6 (300  $\mu$ l)

Concentration: 0.5 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody MT13X3 (150  $\mu$ l)

Concentration: 5  $\mu$ g/ml

**Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (80  $\mu$ l)

**Vial 4**

Recombinant human Thioredoxin-1 standard (0.5  $\mu$ g)

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

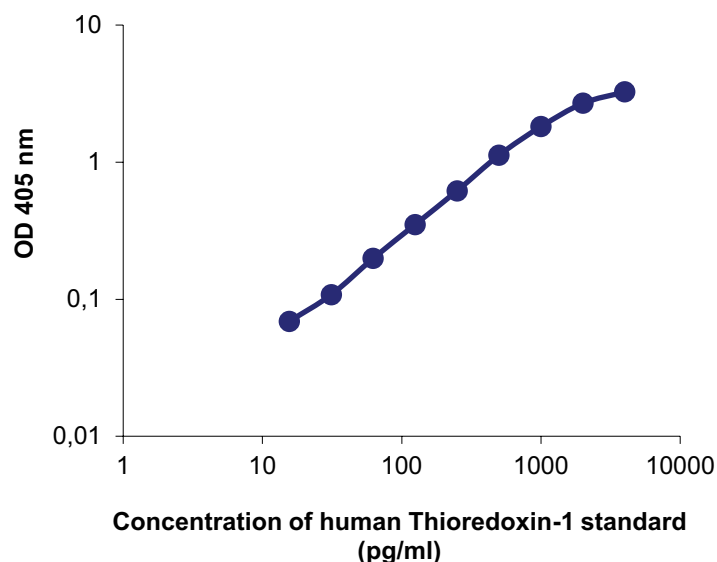
**Intended use:** For quantitative determination of native and recombinant, reduced and oxidized forms of human Thioredoxin-1 (Trx1) in solution, e.g. cell culture supernatant and serum/plasma samples. For accurate measurement of Trx1 blood levels, the use of plasma is recommended since serum contains high levels of Trx1 released from platelets. To minimize the platelet content in plasma, an additional centrifugation at 10,000 x g for 10 minutes in connection with the plasma preparation is recommended. The monoclonal antibodies cross-react with bovine Trx1, thus cell culture supernatants must be free of bovine serum for determination of human Trx1.

**Serum/plasma samples:** Please note that Trx1 determinations in serum/plasma requires the use of Assay buffer (product code: 3652-J2) 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 Assay buffer has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Please contact Mabtech for further information.

**Reagents:** MAb MT17R6 is supplied in sterile-filtered (0.2  $\mu\text{m}$ ) PBS with sodium azide (0.02%) and mAb MT13X3 in sterile-filtered (0.2  $\mu\text{m}$ ) PBS-0.5% BSA with sodium azide (0.02%) . Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.

**Standard range:** 20-2000 pg/ml

**Standard calibration:** No international standard exists for calibration



# Guidelines for Human Thioredoxin-1 ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb MT17R6, diluted to 2  $\mu\text{g}/\text{ml}$  in PBS, pH 7.4, by adding 100  $\mu\text{l}/\text{well}$ . Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200  $\mu\text{l}/\text{well}$ ).
  3. Block plate by adding 200  $\mu\text{l}/\text{well}$  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 Thioredoxin-1 standard by reconstituting contents of vial 4 in 1 ml PBS to a concentration of 0.5  $\mu\text{g}/\text{ml}$ . 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  $\mu\text{l}/\text{well}$  of samples or standards diluted in incubation buffer or Assay buffer for serum/plasma samples and incubate for 2 hours at room temperature.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb MT13X3-biotin at 0.01  $\mu\text{g}/\text{ml}$  in incubation buffer or Assay buffer for serum/plasma samples. Incubate for 1 hour at room temperature.
  9. Wash as in step 4.
  10. Add 100  $\mu\text{l}/\text{well}$  of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
  11. Wash as in step 4.
  12. Add 100  $\mu\text{l}/\text{well}$  of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP), available from Mabtech product code 3652-P10.
  13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



**The products are 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 there from.

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