IMCO
The Expert in Thioredoxin and Glutaredoxin Systems

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PRODUCT SPECIFICATION

Product No.	**AGRX-04**
Product description:	**Antibody (goat) to Human glutaredoxin 1, biotinylated**
Lot No.	**1**
Specifications:	The gamma globulin fraction from goat, immunized with recombinant human glutaredoxin 1 (Grx1) was affinity purified on a column of human Grx1 and biotinylated. Each ampoule (192 µl) contains 50µg of antibody in PBS + 15 mM sodium azide, pH 7.5. The preparation (0.26 mg/ml) comprises inhibitory as well as non-inhibitory antibodies and is useful for ELISA applications. See protocol enclosed
Reference:	Lundberg, M., Potamitou-Fernandes, A., Kumar, S. and Holmgren, A.: Cellular and plasma levels of human glutaredoxin 1 and 2 detected by sensitive ELISA systems. Biochem. Biophys. Res. Commun., 319, 801-809, 2004
Storage:	Keep at +4°C
Examples of use:	ELISA see protocol Good for Western blotting . Good for Immunohistochemistry.
NOTE!	During shipment, the contents of the antibody ampoule may have been redistributed on the inner walls. Before opening the screw-cap of the antibody tube, we recommend to centrifuge the tube shortly, in order to recover liquid from tube cap and walls.

Grx 1 ELISA Protocol

Buffers:
Sodium Carbonate buffer pH 9.7
0.1% Tween in PBS (PBS-T)
PBS, 0.5% BSA, 0.02% NaN_3 (incubation buffer)
10% Diethanolamine, 0.5mM $MgCl_2$, 0.02% NaN_3 pH 9.8 (substrate buffer)

Day 1

1. Dilute anti-Grx1 (goat) to 5 µg/ml in Sodium Carbonate buffer pH 9.7.
2. Coat the wells by adding 50 µl 5 µg/ml anti-Grx1 into the wells.
3. Incubate the plate o/n at 4 °C with slight shake.
4. Prepare sample and Grx1 standard

   Grx1 Standard:

   Prepare 200 µl 50 ng/ml Grx1 in incubation buffer.

   Sample:

   Mix 100 µl sample with 100 µl incubation buffer.
5. Incubate the plate o/n at 4 °C with slight shake.

Day 2

6. Discard the content of the plate.
7. Add 200 µl in the wells and incubate 3 hours at 4 °C with slight shake.
8. Discard the incubation buffer and wash the plate 4x with PBS-T.
9. Add 50 µl incubation buffer in those wells where standards and samples are to be diluted.
10. For standards; add 100 µl in wells A12 and B12, remove 50 µl and add it to the next well and so on.
11. For the sample; add 100 µl in the desired wells, remove 50 µl and add it to the next well and so on.
12. Incubate the plate o/n at 4 °C with slight shake.

Day 3

13. Discard the content of the plate and wash the plate 4x with PBS-T.
14. Dilute biotinylated anti-Grx1 (goat) to 1 µg/ml in incubation buffer and add it to the wells.
15. Incubate 2 hours at room temperature with slight shake.
16. Discard the content of the plate and wash the plate 4x with PBS-T.
17. Dilute streptavidin-ALP 1:1000 in incubation buffer, add 50 µl to each well incubate for 1 hour at room temperature with slight shake.
18. While incubating; add 1 tablet 4-nitrophenyl phosphate in 5 ml substrate buffer.
19. Discard the content of the plate and wash the plate 6x with PBS-T.
20. Add 50 µl 4-nitrophenyl phosphate to the wells.
21. Incubate at least 15 min (sometimes the reaction takes longer)
22. Read A_{405}.