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PRODUCT SPECIFICATION

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| Product No. | TR-03B |
| Product description: | Rat Recombinant Thioredoxin Reductase 1 |
| Lot No. | 20 |
| Specifications: | <p>One ampoule contains 100 µl of recombinant rat thioredoxin reductase 1 (TrxR1) in 50 mM Tris-HCl, 1 mM EDTA, pH 7.5 and 50 percent glycerol with a total activity of 5 units and 0.15 mg of active enzyme protein. One unit of enzyme catalyses oxidation of one micromol of NADPH per min under standard mammalian TrxR assay conditions in the presence of 5 mM DTNB. The specific activity of this preparation is 40 U/mg (see Qing and Arner below).</p> <p>Use: Add 0.90 ml of 50 mM Tris-HCl- 1 mM EDTA, pH 7.5 (or other buffer with EDTA) and mix. This will give 1 ml of a solution with 5 Units /ml or 2.8 µM active enzyme.</p> |
| References: | <p>Cheng, Q and Arner ESJ : Selenocysteine insertion at a predefined UAG codon in a release factor 1 (RF1)-depleted <i>Escherichia coli</i> host strain bypasses species barriers in recombinant selenoprotein translation. (2017), J. Biol. Chem 292, 5476-5487.</p> <p>Holmgren A. and Björnstedt, M. (1995) Methods Enzymol, 252, 199-208.</p> |
| Storage: | Keep enzyme frozen in aliquots at -20° C in the dark. |
| Examples of use: | <p>Activity can be tested with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as substrate using 5 mM DTNB and 0.2 mM NADPH in 0.1 M potassium phosphate, 1 mM EDTA, pH 7.0 plus 0.1 mg/ml bovine serum albumin. Mammalian thioredoxin reductase at a concentration of 0.92 nM gives a change in absorption at 412 nm of 0.100 per min in a 1 cm cuvette. K_{cat} with DTNB is 3000 min⁻¹.</p> <p>Activity can alternatively be measured with the thioredoxin-dependent reduction of insulin using either human thioredoxin (K_m 2.5 µM, Product No TRX-03) or <i>E.coli</i> thioredoxin (K_m 35 µM, Product No TRX-01B). The test mixture should contain 160 µM insulin and 0.2 mM NADPH in 0.1 M potassium phosphate, 2mM EDTA, pH 7,0. In the presence of 5 µM human thioredoxin, 7 nM of mammalian thioredoxin reductase will give a decrease of absorbtion at 340 nm of 0.100 min⁻¹. In case of 5 µM <i>E.coli</i> thioredoxin, 7 nM of the enzyme will give a decrease of absorbance at 340 nm of 0.017 min⁻¹. For assays of Trx use 50 or 100 nM enzyme.</p> |
| NOTE! | <p>Some solution may stick to the rubber cover or stay on the walls of the vial. This enzyme is expressed in <i>E.coli gor</i> mutant and is thus free of glutathione reductase activity. Pure thioredoxin reductase is 35 Units per mg protein and has a molecular mass of 112 000 with two identical subunits. Due to UGA codon acting as stop this preparation contains about 50 percent of the truncated protein lacking the Sec-Gly C-terminus. It is homogenous on Western blots. The truncated protein is folded and does not influence activity.</p> |

