

## Supplementary Material

### *Construction of 6xHis-tagged Gpx1*

The *GPX1* gene was amplified by PCR using the primers GPX1-Nde, 5'-TATTTATAAGTCCATATGCAAGAAT-3'; and GPX1-Bam, 5'-AAAATGACTGGATCCTACATATTCTT-3', and chromosomal DNA of YPH250 as a template. *NdeI* and *BamHI* sites were designed in GPX1-Nde and GPX1-Bam, respectively (underlined). The PCR fragment was digested with *NdeI* and *BamHI*, and the resultant fragment was cloned into the *NdeI-BamHI* site of the vector pET-15b (Novagen). The chimeric plasmid (pET15b-GPX1) was introduced into *E. coli* BL21 (DE3).

### *Substitution of cysteine in Gpx1*

The *GPX1* mutants carrying a cysteine-to-serine substitution were created by PCR-mediated site-directed mutagenesis (Ho *et al.*, 1989) using the following primers:

GPX1-HA-F,	5'-AGTTAA <u>ACTCGAGT</u> ATTGGATCCTATAAGG-3';	GPX1-HA-R,
5'-TAAAGATCTTCTTCTGGTGGTTGATTCAGT-3';		GPX1-C36S-F,
5'-GCATCTCATTCTGCGTTCACACCTC-3';		GPX1-C36S-R,
5'-TGTGAACGCAGAATGAGATGCTACA-3';		GPX1-C64S-F,
5'-GCCTTTCCTCTGGTCAATTCGGAA-3';		GPX1-C64S-R,
5'-GAATTGACCAGAGGGAAAGGCCACA-3';		GPX1-C82S-F,
5'-AATAAGTTCTCTCAAGATAAATATG-3';		GPX1-C82S-R,

5'-TTTATCTTGAGAGAACTTATTAATC-3'; GPX1-C98S-F,  
5'-AAGATCCGTTCCAACGGGCAAAAGC-3'; GPX1-C98S-R,  
5'-TTGCCCGTTGGAACGGATCTTATGT-3'; GPX1-C144S-F,  
5'-AGGTTTTTCATCCATGACTAGGCCAC-3'; GPX1-C144S-R,  
5'-CCTAGTCATGGATGAAAACCTTTTA-3'; GPX1-C152S-F,  
5'-CTAGAGCTTTCTCCAATAATCGAAG-3'; and GPX1-C152S-R,

5'-GATTATTGGAGAAAGCTCTAGTGGC-3'. A *XhoI* site and a *BglII* site (underlined) were designed in GPX1-HA-F and GPX1-HA-R, respectively. To create the mutation in Cys<sup>36</sup>, the first PCR was done with GPX1-HA-F plus GPX1-C36S-R, and GPX1-C36-F plus GPX1-HA-R, and chromosomal DNA of YPH250 as a template. The second PCR was done with GPX1-HA-F plus GPX1-HA-R, and a mixture of the first PCR products. The resultant PCR fragment was digested with *XhoI* and *BglII*, and the DNA fragment was inserted in the *XhoI-BglII* site of pSLF172, which is designed to add three copies of the HA (hemagglutinin) tag at the carboxy terminus of the gene of interest (Forsburg and Sherman, 1997). The resultant plasmid was named pSLF172-GPX1<sup>C36S</sup>-3HA. Similarly, a series of plasmids for cysteine-substituted *GPX1* mutants (pSLF172-GPX1<sup>C64S</sup>-, -GPX1<sup>C82S</sup>-, -GPX1<sup>C98S</sup>-, -GPX1<sup>C144S</sup>-, and -GPX1<sup>C152S</sup>-3HA) were created.

To construct the pRS416 (*CEN*-type plasmid)-based plasmid, each pSLF172-GPX1-3HA plasmid was digested with *SalI* and *HindIII*, and the DNA fragment containing *GPX1-3HA* was cloned to the *HindIII-SalI* site of pRS416. The resultant plasmids were named pRS416-GPX1<sup>C64S</sup>-, -GPX1<sup>C82S</sup>-, -GPX1<sup>C98S</sup>-, -GPX1<sup>C144S</sup>-, and -GPX1<sup>C152S</sup>-3HA.

### **AMS assay**

Modification of cysteine residues of Gpx1 with 4'-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS) (Sigma) was done according to our previous method (Tanaka *et al.*, 2005). After modification, Gpx1-3HA protein was separated with non-reducing SDS-PAGE followed by Western blotting using anti-HA monoclonal antibody (Cell Signaling).

To determine the redox status of Gpx1 produced in *E. coli*, cell lysates (protein concentration, 1 mg /ml) containing His-tagged Gpx1 were treated with 5 mM DTT or 1 mM H<sub>2</sub>O<sub>2</sub> (total volume, 100 µl) for 60 min at 25°C. Proteins were precipitated by trichloroacetic acid solution followed by washing the pellets with acetone. The dried materials were treated with AMS (total volume, 70 µl), and 5µl (3.5 µg protein) was subjected to non-reducing SDS-PAGE followed by Western blotting using anti-His-tag antibody (laboratory stock).

### **References**

- Forsburg SS & Sherman DA (1997) General purpose tagging vectors for fission yeast. *Gene* **191**: 191-195.
- Ho SN, Hunt HD, Horton RM, Pullen JK & Pease LR (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77**: 51-59.
- Tanaka T, Izawa, S & Inoue Y (2005) *GPX2*, encoding a phospholipid hydroperoxide glutathione peroxidase homologue, codes for an atypical 2-Cys peroxiredoxin in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**: 42078-42087.