



## Product Information

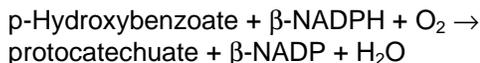
### p-Hydroxybenzoate Hydroxylase from *Pseudomonas sp.*

Product Number **H 9886**  
Storage Temperature -20 °C

#### Product Description

Enzyme Commission (EC) Number: 1.14.13.2  
CAS Number: 9059-23-8  
Molecular Weight: 83.6 kDa<sup>1</sup>  
Synonyms: 4-hydroxybenzoate, NADPH:oxygen  
oxidoreductase (3-hydroxylating)

p-Hydroxybenzoate hydroxylase is a flavoprotein containing one mole of FAD per mole of protein. This enzyme constitutes a key enzyme in the  $\beta$ -ketoacid pathway in bacteria.<sup>1-3</sup> p-Hydroxybenzoate hydroxylase catalyzes the following reaction:



p-Hydroxybenzoate inserts molecular oxygen into aromatic rings to form phenols. The oxygen molecule initially reacts with FAD to form a derivative of FAD, thought to be peroxyflavin. This intermediate then decays either to oxidized enzyme bound flavin and H<sub>2</sub>O<sub>2</sub> or transfers an atom of oxygen to the aromatic substrate, depending upon the aromatic compound bound to the enzyme.<sup>4</sup>

The enzyme is highly specific for p-hydroxybenzoate with a K<sub>m</sub> of 0.021 mM. The K<sub>m</sub> for  $\beta$ -NADPH is 0.023 mM. The enzyme is active between the pH range of 7.0 - 9.0, with a pH optimum at pH 8.0.<sup>1</sup>

p-Hydroxybenzoate hydroxylase is a sulfhydryl containing enzyme and is inhibited by p-hydroxymercuribenzoate (0.5 mM) and Zn<sup>2+</sup> (0.2 mM). This inhibition can be largely reversed (65% and 94%) by the presence of 10 mM glutathione.<sup>1,5</sup>

#### Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

#### Preparation Instructions

This enzyme is soluble in 50 mM potassium phosphate buffer, pH 6.0 (0.5 mg/ml), yielding a clear solution.

#### References

- Hosokawa, K., and Stanier, R.Y., Crystallization and properties of p-hydroxybenzoate hydroxylase from *Pseudomonas putida*. *J. Biol. Chem.*, **241(10)**, 2453-2460 (1966).
- Hosokawa, K., Regulation of synthesis of early enzymes of p-hydroxybenzoate pathway in *Pseudomonas putida*. *J. Biol. Chem.*, **245(20)**, 5304-5308 (1970).
- Bertani, I., et al., Regulation of the p-hydroxybenzoic acid hydroxylase gene (pobA) in plant growth promoting *Pseudomonas putida* WCS258. *Microbiology*, **147(Pt 6)**, 1611-1620 (2001).
- Entsch, B., et al., Flavin-oxygen derivatives involved in hydroxylation by p-hydroxybenzoate hydroxylase. *J. Biol. Chem.*, **251(9)**, 2550-2563 (1976).
- Van Berkel, W.J., et al., Chemical modification of sulfhydryl groups in p-hydroxybenzoate hydroxylase from *Pseudomonas fluorescens*. Involvement in catalysis and assignment in the sequence. *Eur. J. Biochem.*, **145(2)**, 245-256 (1984).

TMG/NSB 1/04

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.