

## Product Information

### Cyclooxygenase 2, human recombinant, expressed in Sf 21 cells

Catalog Number **C0858**

Storage Temperature  $-70\text{ }^{\circ}\text{C}$

EC 1.14.99.1

Synonyms: COX-2, Inducible Cyclooxygenase,  
Prostaglandin H Synthase 2

#### Product Description

COX-2 catalyzes the conversion of arachidonic acid to prostaglandin H<sub>2</sub> (the first step in the biosynthesis of prostaglandins, thromboxanes, and prostacyclins). Prostaglandin H<sub>2</sub> is converted by other enzymes into inflammatory mediators such as prostaglandin (PG) D<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , PGI<sub>2</sub>, and thromboxane A<sub>2</sub>. Thus, COX is a key enzyme in the production of inflammatory agents and is the target of intense research and drug discovery activities.

COX consists of two isoforms, COX-1 (599 amino acid residues) and COX-2 (604 amino acid residues). The COX enzymes, membrane-associated heme proteins that have cyclooxygenase and peroxidase activities, are targets of NSAID (non-steroidal anti-inflammatory drugs) such as aspirin.<sup>1</sup>

COX-2, the inducible form of cyclooxygenase, is induced by phorbol esters, lipopolysaccharides, and cytokines. It appears to be responsible for the biosynthesis of prostaglandins under acute inflammatory conditions.<sup>2</sup>

This human, recombinant COX-2 product, containing a six histidine sequence near the N-terminus, is isolated from a baculovirus overexpression system in Sf 21 cells.<sup>3</sup>

Molecular mass: homodimer (70–74 kDa/subunit)

K<sub>m</sub>:<sup>3</sup> 6.5  $\mu\text{M}$  (arachidonate, exhibits enzyme activity and sensitivity to NSAIDs similar to the non-tagged enzyme)

This COX-2 product is supplied as a solution in 80 mM Tris-HCl, pH 8.0 with 0.1% TWEEN® 20, 300  $\mu\text{M}$  diethyldithiocarbamate, and 10% glycerol.

**Note:** If this preservative is undesirable, it can be removed by standard desalting procedures, but the enzyme is unstable in the absence of the preservative and so should be used within one hour.

Purity:  $\geq 70\%$  (SDS-PAGE)

Specific Activity:  $\geq 8,000$  units/mg protein

Unit Definition: One unit will consume 1.0 nanomole of oxygen per minute at pH 8 at 37  $^{\circ}\text{C}$ .

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

COX-2 contains Fe<sup>3+</sup>-protoporphyrin IX as a cofactor, which may dissociate from the protein during its purification, resulting in a mixture of apo- and holo-enzymes. Therefore, add hematin to the reaction mixture (1  $\mu\text{M}$  final concentration) in order to obtain maximal enzyme activity.

#### Storage/Stability

The product should be stored at  $-70\text{ }^{\circ}\text{C}$ .

COX is relatively unstable at room temperature; to prevent loss of activity during experiments, keep the enzyme on ice (0  $^{\circ}\text{C}$  to 4  $^{\circ}\text{C}$ ) at all times. After initial defrost, it is recommended that the product be aliquoted into tubes and re-frozen at  $-70\text{ }^{\circ}\text{C}$  to avoid repeated freeze-thaw cycles. Do not store in a frost-free freezer.

## References

1. O'Neill, G.P. et al., Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. *Mol. Pharm.*, **45**, 245-254 (1994).
2. Xie, W. et al., Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. USA*, **88**, 2692-2696 (1991).
3. Smith, T. et al., Purification and characterization of the human recombinant histidine-tagged prostaglandin endoperoxide H synthases-1 and -2. *Arch. Biochem. Biophys.*, **375**, 195-200 (2000).

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