Flavin-Containing Monooxygenase 3
human, recombinant
microsomes

Product Number F 5053
Storage Temperature −70 °C

E.C. 1.14.13.8
Synonym: FMO3

Product Description
The microsomal product is prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus (Autographa californica) containing cDNA inserts for the human flavin-containing monoxygenase isozyme. The microsomes are isolated from the endoplasmic reticulum of cultured cells. The recombinant FMO3 has the same mobility (Western immunoblot) as FMO3 in human liver microsomes.

The FMO enzymes metabolize xenobiotic substrates, converting the substrate to a more polar product, aiding in its excretion from the body. They demonstrate a broad substrate specificity and have considerable overlap in function with the cytochrome P450 enzymes. The FMO oxygen transfer potential is much lower than that of the cytochrome P450 enzymes, limiting FMO substrates to heteroatom (nitrogen or sulfur) nucleophiles with the oxidative reaction resulting in the formation of N-oxides and S-oxides, respectively. Substrates for FMO enzymes are typically lipophilic compounds, while charged nucleophiles are poor FMO substrates. The FMO mechanism differs from that of cytochrome P450 enzymes by binding and activating molecular oxygen prior to binding of the substrate. NADPH binds to the FMO enzyme and reduces the FAD prosthetic group to FADH₂. The NADP⁺ remains bound to the enzyme and molecular oxygen binds to the prosthetic group and forms the reactive peroxylavin species. Monoxygenation of the substrate and release of a molecule of water leaves the flavin in its oxidized state. The NADP⁺ molecule dissociates and the FMO enzyme is ready for another catalytic cycle. This mechanism has a single two electron reduction step compared to the cytochrome P450 enzyme mechanism, which has two separate one electron reduction steps.

The monooxygenase activity of both FMO and cytochrome P450 enzymes is responsible for oxidative metabolism of many drugs in body. The study of these enzymes and the metabolites formed is important for they often affect the biological lifetime, toxicity, and pharmacological properties of the drug of interest.

FMO enzymes are classified into 5 single member subfamilies categorized as FMO1-5, which have approximately 50-60% amino acid sequence homology. The FMO enzymes have a molecular mass range of 55 to 60 kDa and have been isolated from a variety of tissues including lung, kidney, and liver. The presence and concentration of a specific isozyme in a given tissue varies broadly from species to species, and in some cases, by gender (observed in mice and dogs). In humans, FMO1 is found in the kidney and fetal liver. In human adults FMO1 is replaced by FMO3 as the primary human liver isozyme. FMO5 is minimally effective as a drug metabolizing enzyme. Although this isozyme has been isolated in a multitude of tissues, only a limited number of substrates have been identified.

The product is supplied in a solution of 100 mM potassium phosphate buffer, pH 7.4. Protein content, methyl p-tolyl sulfide oxidase activity, and FAD content¹ are reported on a lot-to-lot basis.

Precautions and Disclaimer
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
1. Quickly thaw at 37 °C using a water bath. Keep on ice until ready to use.
2. If not using the entire contents, aliquot to minimize freeze-thaw cycles. Generally, 80% or more of the activity is retained after 6 freeze-thaw cycles.
3. Store aliquots at −70 °C.
Storage/Stability
The product is shipped on dry ice and it is recommended to store the product at −70 °C. The product as supplied is stable for at least 2 years.

Procedure
Methyl p-Tolyl Sulfide Oxidase Activity:
A 0.25 ml reaction containing the following was incubated at 37 °C for 10 minutes:
- 50 µg FMO3 microsomal protein
- 0.065 mM NADP⁺
- 3.3 mM glucose-6-phosphate
- 0.4 unit/ml glucose-6-phosphate dehydrogenase
- 3.3 mM MgCl₂
- 2.0 mM methyl p-tolyl sulfide (Product No. 27,595-6)
- 0.05 M glycine buffer, pH 9.5

The reaction was stopped with 75 µl of acetonitrile and centrifuged (10,000 x g) for 5 minutes. A 50 µl aliquot of the supernatant was chromatographically separated by HPLC. The product was detected by measuring absorbance at 237 nm and comparison was made to a standard curve of methyl p-tolyl sulfoxide (Product No. 48,185-8).

Notes: With respect to enzyme concentration, catalysis is linear up to at least 0.5 mg/ml. The oxidation of methyl p-tolyl sulfide is approximately linear for 30 minutes. Other substrates may not exhibit similar linearity. NADPH may be substituted for the NADPH generating system, which consists of NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase.

References

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