Discovery Life Sciences 6 Henshaw Street Woburn, MA 01801

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Human FMO3 SUPERSOMES[™]

Catalog Number.....456233 Lot Number......2404120 Storage Conditions..STORE AT -80°C Date Released2024 August Expiration Date......2034 April

Package Contents	2.5 mg protein in 0.5 ml
Protein Content	
Methyl p-Tolyl Sulfide	
Oxidase Activity	
FAD Content ¹	

This activity is catalyzed by FMO3 which is expressed from human FMO3 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. A microsome preparation using wild type virus (Catalog No. 456201) should be used as a control for native activities.

METHOD: A 0.25 ml reaction mixture containing 50 μ g protein, 0.065 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride, and 2.0 mM methyl p-tolyl sulfide in 0.05 M glycine (pH 9.5) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 75 μ l acetonitrile and centrifuged (10,000 x g) for 5 minutes. 50 μ l of the supernatant was injected into a 4.6 x 250 mm 5 μ C18 HPLC column and separated at 45°C with a mobile phase initially of 46% methanol increasing to 55% methanol over 7 min. (the substrate was then eluted with 100% methanol) and at a flow rate of 1.0 ml per min. The product was detected by its absorbance at 237 nm and quantitated by comparing the absorbance to a standard curve of methyl p-tolyl sulfoxide.

ADVICE

- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- Aliquot to minimize freeze-thawing cycles. Less than 20% thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 0.5 mg per ml.
- Metabolite production with methyl p-tolyl sulfide is approximately linear for 30 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.
- Western immunoblotting indicates the expressed FMO3 has the same mobility as FMO3 in human liver microsomes.



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¹ FAD content was determined by a method based on the one described by Lang et al. in Biochemical Pharmacology **56**, 1005 (1998).

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Safety Recommendations:

Safety assessment indicates this product is hazardous; refer to SDS [Safety Data Sheet] for safety information. Handle in accordance with good industrial hygiene and laboratory safety practices.

HAZARD WARNING:

The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

SAFETY RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

Do not eat, drink or smoke.

Avoid contact with skin or eyes.

Do not inhale aerosols.

Do not pipette by mouth.

Wear suitable protective clothing, gloves and eye protection.

Steam sterilize product or treat product with a 1% solution of sodium hypochlorite prior to disposal.

Alexa Silies

08/27/2024

Quality Assurance

Date

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Revision History					
Rev.	Change No.	Description of Change	Revised By	Revised Date	
1	CC-02544	Updated to Corning branding, protected variable fields, replace safety section with safety statement and clarify SDS [Safety Data Sheet] information, addition of QA signature and date	L. Brown	7/23/14	