



## Product Information

### Microsomes, pooled from CMV negative human liver

Product Number **M 0442**  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

#### Product Description

Liver microsomes are subcellular particles derived from the endoplasmic reticulum of hepatic cells. These microsomes are a rich source of drug metabolizing enzymes, including cytochrome P450. Microsome pools from various sources are useful in the study of xenobiotic metabolism and drug interactions.

This product contains a mixture of liver microsomes pooled from different individual human donors. The pathogenicity testing of all liver specimens has been performed using a PCR protocol. The donors are human of mixed age. The donors are in various states of health, however each liver tested negative for CMV, HIV1&2, HTLV1&2, and hepatitis B and C.

The protein content is a minimum of 20 mg/ml in 250 mM sucrose and is specifically reported on the certificate of analysis (C of A). Total cytochrome P450, oxidoreductase, cytochrome  $b_5$ , CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A, and FMO activities are also reported on the lot specific C of A.

#### 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  $37\text{ }^{\circ}\text{C}$  using a water bath. Keep on ice until ready to use.
2. If not using the entire contents, aliquot to minimize freeze-thaw cycles.
3. Store aliquots at  $-70\text{ }^{\circ}\text{C}$ .

#### Storage/Stability

The product is shipped on dry ice and it is recommended to store the product at  $-70\text{ }^{\circ}\text{C}$ . The product, as supplied, is stable for at least 2 years if stored properly.

#### Product Profile

Total cytochrome P450 and cytochrome  $b_5$  are assayed by the standard method of Omura and Sato.<sup>1</sup>

Enzyme activities on the product are determined as follows:

##### Oxidoreductase Activity:

Determined as Cytochrome c reductase activity. The reaction is initiated by the addition of 0.1 mg/ml protein to 1.0 ml of reaction mixture containing 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, 3.3 mM  $\text{MgCl}_2$ , and 0.95 mg/ml cytochrome c in 0.25 M potassium phosphate buffer, pH 7.4, at  $37\text{ }^{\circ}\text{C}$ . The absorbance change at 550 nm is recorded as a function of time. An extinction coefficient for reduced (ferrous) cytochrome c at 550 nm of  $19.6\text{ mM}^{-1}\text{ cm}^{-1}$  is used to calculate the reductase activity. One unit will reduce 1 nanomole of cytochrome c per minute at pH 7.4 at  $37\text{ }^{\circ}\text{C}$ .

##### CYP1A2 Isozyme Activity:

Determined as phenacetin O-deethylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM  $\text{MgCl}_2$  in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of acetamidophenol per minute at pH 7.4 at  $37\text{ }^{\circ}\text{C}$ .

##### CYP2D6 Isozyme Activity:

Determined as bufuralol 1'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM  $\text{MgCl}_2$  in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 1'-hydroxybufuralol per minute at pH 7.4 at  $37\text{ }^{\circ}\text{C}$ .

#### CYP2E1 Isozyme Activity:

Determined as chlorzoxazone 6-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 6'-hydroxychlorzoxazone per minute at pH 7.4 at 37 °C.

#### CYP2A6 Isozyme Activity:

Determined as coumarin 7-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M Tris, pH 7.5, for 20 minutes. One unit will produce 1 picomole of 7-hydroxycoumarin per minute at pH 7.5 at 37 °C.

#### CYP2B6 Isozyme Activity:

Determined as (S)-mephenytoin N-demethylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.05 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of nervalol per minute at pH 7.4 at 37 °C.

#### CYP2C19 Isozyme Activity:

Determined as (S)-mephenytoin 4'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.05 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 4'-hydroxymephenytoin per minute at pH 7.4 at 37 °C.

#### CYP2C8 Isozyme Activity:

Determined as paclitaxel 6 $\alpha$ -hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M potassium phosphate buffer, pH 7.4, for 10 minutes. One unit will produce 1 picomole of 6 $\alpha$ -hydroxypaclitaxel per minute at pH 7.4 at 37 °C.

#### CYP2C9 Isozyme Activity:

Determined as diclofenac 4'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M Tris, pH 7.5, for 10 minutes. One unit will produce 1 picomole of 4'-hydroxydiclofenac per minute at pH 7.5 at 37 °C.

#### CYP4A Isozyme Activity:

Determined as lauric acid 12-hydroxylase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M Tris, pH 7.5, for 10 minutes. One unit will produce 1 picomole of 12-hydroxylauric acid per minute at pH 7.5 at 37 °C.

#### CYP3A4 Isozyme Activity:

Determined as testosterone 6 $\beta$ -hydroxylase activity. Incubations are conducted at 0.5 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub> in 0.1 M potassium phosphate buffer, pH 7.4, for 10 minutes. One unit will produce 1 picomole of 6 $\beta$ -hydroxytestosterone per minute at pH 7.4 at 37 °C.

#### FMO Activity:

Determined as methyl p-tolyl sulfide oxidase activity. Incubations are conducted at 0.8 mg/ml of protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl<sub>2</sub>, 1.2 mM diethylenetriaminepentacetic acid, 0.5 mg/ml TRITON<sup>®</sup> X-100 in 0.05 M glycine buffer, pH 9.5, for 10 minutes. One unit will produce 1 picomole of methyl p-tolyl sulfoxide per minute at pH 9.5 at 37 °C.

#### **References**

1. Omura, T., and Sato, R., J. Biol. Chem., **239**, 2379, (1964).

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