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ProductInformation

Microsomes, pooled
from female mouse liver (CD-1)

Product Number **M 9566**
Storage Temperature –70 °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 female mice (CD-1) of 11 weeks of age.

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, CYP1A, CYP2E, CYP3A, and CYP4A 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 °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 °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 if stored correctly.

Product Profile

Total cytochrome P450 and cytochrome b_5 are assayed by the standard method of Omura and Sato.[1]

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 of 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 $MgCl_2$, and 0.95 mg/ml cytochrome c in 0.25 M potassium phosphate buffer, pH 7.4, at 37 °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 mM^{-1} 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 °C.

CYP1A Isozyme Activity:
Determined as 7-ethoxyresorufin O-deethylase 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 $MgCl_2$ in 0.1 M potassium phosphate buffer, pH 7.4, for 2 minutes. One unit will produce 1 picomole of resorufin per minute at pH 7.4 at 37 °C.

CYP2E Isozyme Activity:
Determined as p-nitrophenol hydroxylase activity. Incubations are conducted at 0.2 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 $MgCl_2$ in 0.1 M potassium phosphate buffer, pH 7.4, for 30 minutes. One unit will produce 1 picomole of 4-nitrocatechol per minute at pH 7.4 at 37 °C.

CYP3A Isozyme Activity:
Determined as testosterone 6β-hydroxylase activity. Incubations are conducted at 0.2 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 $MgCl_2$ in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 6β-hydroxytestosterone per minute at pH 7.4 at 37 °C.

CYP4A Isozyme Activity:
Determined as [^{14}C] lauric acid 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 $MgCl_2$ in 0.1 M Tris buffer, 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.

References

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

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