

Sacchrosomes - Human Cytochrome P450s in a Yeast Expression System.

Human CYP1A2 + P450 Reductase

Product overview

Catalogue Number CYP1A2-1 Lot Number 1A2-10-07

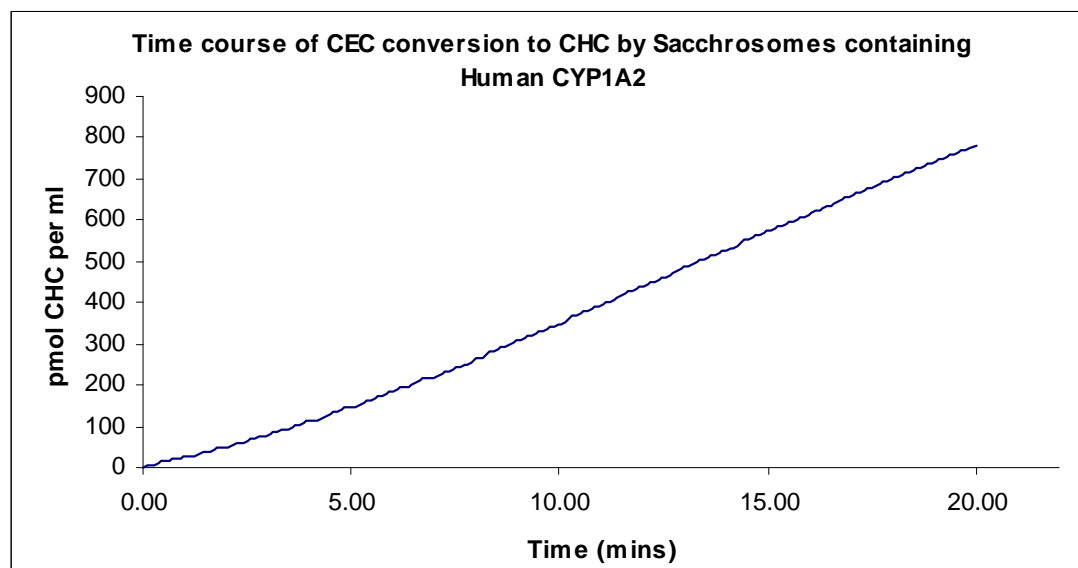
Human CYP1A2 is a major hepatic cytochrome P450 and is implicated in the metabolism of drugs such as clozapine, theophylline and tacrine.

Membranes consist of Human Cytochrome P450 1A2 and P450 Reductase enzymes bound within a yeast microsomal fraction.

Product Data

Pack Size	0.5 nmol
Volume per tube	0.5 ml
Cytochrome P450 Content	116 pmol.mg ⁻¹
Protein Concentration	8.62 mg.ml ⁻¹
Specific Activity	3 pmol CHC.min ⁻¹ . pmol P450 ⁻¹
Cytochrome P450 Reductase Activity	1051 nmol MTT red. min ⁻¹ .mg protein ⁻¹

Fluorometric assay of CYP1A2- Graph depicts product formation (3-cyano-7-hydroxycoumarin) in pmol over time in min.



Specific Activity

CYP1A2 activity assay performed in a microplate-based fluorometric assay with 3-cyano-7-ethoxycoumarin (CEC) as a substrate in a 0.1M phosphate buffer (pH 7.4). Excitation at 400nm and Emission at 460nm, temperature held at 37°C.

0.1 ml of reaction mix contains 1.3mM NADP⁺, 3.3 mM Glucose-6-phosphate, 3.3 mM Magnesium Chloride, 0.04U of Glucose-6-phosphate dehydrogenase and 16 µM of CEC. 1.0 pM of Sacchrosome 1A2 is added per reaction.

The conversion of 3-cyano-7-ethoxycoumarin substrate to 3-cyano-7-hydroxycoumarin (CHC) product is measured over time. Values converted using a standard curve of 3-cyano-7-hydroxycoumarin.

Cytochrome P450 Content

CO binding assay performed in a cuvette format using a dual beam spectrophotometer scanning from 500 to 400nm. Spectral difference of microsomes measured in a phosphate glycerol buffer with the addition of sodium dithionite with and without CO perfusion.

Cytochrome P450 Reductase Activity

Reduction of MTT by Cytochrome P450 Reductase utilising a regenerating system in a phosphate buffer was measured over time.

Protein Concentration

Total protein was measured using a microplate-based Bradford assay method with BSA as a standard.

Product Use

For best stability thaw on ice, aliquot suitable quantities for your studies and store at -80°C.

Microsomes are supplied in a buffer containing Water, Tris, EDTA and Glycerol which are unlikely to interfere with most assays.

Studies indicate product stability at -80°C for at least 12 months.

Safety

This product is not suspected to contain any pathogenic or hazardous materials. However, since these properties have not been investigated handle with care in accordance to your normal laboratory practices.

This product is only intended for *in vitro* research use and is not licensed as a drug, therapeutic or diagnostic tool for humans or animals.

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