

## Sacchrosomes - Human Cytochrome P450s in a Yeast Expression System.

# Human CYP2A6 + P450 Reductase + Cytochrome b<sub>5</sub>

### *Product overview*

Catalogue Number CYP2A6-1      Lot Number 2A6-10-07

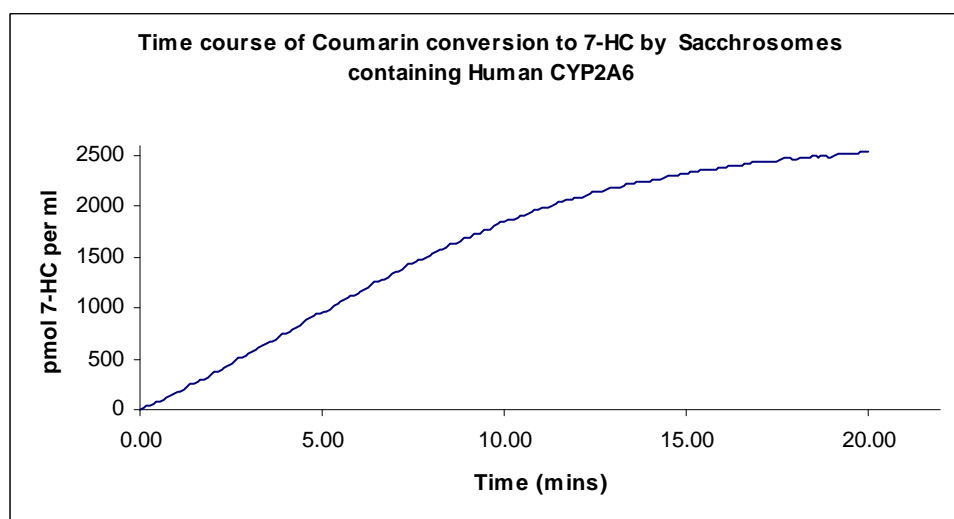
Human CYP2A6 is expressed predominantly in the hepatic tissues and is known to be induced in the liver by phenobarbital and rifampicin. It is the only enzyme in the human body that appreciably catalyzes the 7-hydroxylation of coumarin.

Membranes consist of Human Cytochrome P450 2A6, P450 Reductase enzymes and Cytochrome b<sub>5</sub> bound within a yeast microsomal fraction.

### *Product Data*

Pack Size	0.5 nmol
Volume per tube	0.710 ml
Cytochrome P450 Content	44 pmol.mg <sup>-1</sup>
Protein Concentration	16.1 mg.ml <sup>-1</sup>
Specific Activity	19.16 pmol 7-HC.min <sup>-1</sup> . pmol P450 <sup>-1</sup>
Cytochrome P450 Reductase Activity	1258 nmol MTT reduced.min <sup>-1</sup> .mg protein <sup>-1</sup>
Cytochrome b <sub>5</sub> content	0.45 nmol. mg <sup>-1</sup>

**Fluorometric assay of CYP2A6- Graph depicts product formation (7-hydroxycoumarin) in pmol over time in min.**



### **Specific Activity**

CYP2A6 activity assay performed in a microplate-based fluorometric assay with Coumarin substrate in 100mM Tris buffer (pH 7.5). Excitation at 400nm and Emission at 460nm, temperature held at 37°C.

0.1 ml of reaction mix contains 0.065mM NADP<sup>+</sup>, 3.3 mM Glucose-6-phosphate, 3.3 mM Magnesium Chloride, 0.04U of Glucose-6-phosphate dehydrogenase and 3 µM of Coumarin. 1.0 pM of Sacchosome 2A6 is added per reaction

The conversion of the coumarin substrate to 7-hydroxycoumarin (7-HC) product is measured over time. Values converted using a standard curve of 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.

### **Cytochrome b5 Activity**

Cytochrome b5 assay performed in a cuvette format using a dual beam spectrophotometer scanning from 500 to 400nm. Spectral differences of microsomes were measured in phosphate buffer. Sample is reduced by sodium dithionite and compared against oxidised sample.

### **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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