

Sacchrosomes - Human Cytochrome P450s in a Yeast Expression System.

Human CYP3A4 + P450 Reductase + Cytochrome b₅

Product overview

Catalogue Number CYP3A4-1 Lot Number 3A4-10-07

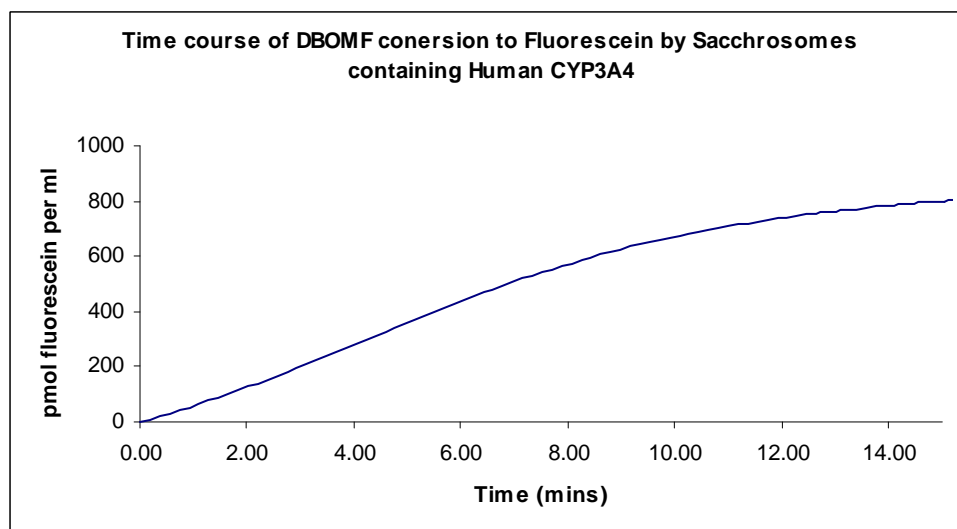
The Human CYP3A4 enzymes is one of the most important inducible drug metabolism enzyme and is present in both hepatic and extra hepatic tissues. This enzyme can metabolise 40-45% of all clinically used drugs.

Membranes consist of Human Cytochrome P450 3A4, P450 Reductase enzymes and Cytochrome b₅ bound within a yeast microsomal fraction.

Product Data

Pack Size	0.5 nmol
Volume per tube	0.545 ml
Cytochrome P450 Content	42.0 pmol.mg ⁻¹
Protein Concentration	21.8 mg.ml ⁻¹
Specific Activity	7.6 pmol Fluorescein.min ⁻¹ . nmol P450 ⁻¹
Cytochrome P450 Reductase Activity	849 nmol MTT reduced.min ⁻¹ .mg protein ⁻¹
Cytochrome b ₅ content	0.411 nmol. mg ⁻¹

Fluorometric assay of CYP3A4- Graph depicts product formation (Fluorescein) in pmol over time in min.



Specific Activity

CYP3A4 activity assay performed in a microplate-based fluorometric assay with DBOMF as a substrate in 0.1M phosphate buffer (pH 7.4). Excitation at 485nm and Emission at 528nm, 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 2.0 µM of DBOMF. 1.0 pM of Sacchrosome 3A4 is added per reaction
The conversion of DBOMF substrate to fluorescein product is measured over time.
Values converted using a standard curve of fluorescein.

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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