

HUMAN CYTOCHROME P450 2C9 (CYP2C9) YR

ORDERING INFORMATION

Catalogue Number: M40009

Size: 1 nmol

Stability: ≥ 2 years at -80 °C

Storage: -80 °C
Avoid frequent temperature changes
Thaw on ice

Shipping: dry ice

PRODUCT DESCRIPTION

Microsome contents: Human CYP2C9 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*

Storage buffer: 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol

BATCH XXXX (below typical batch characteristics)

P450 concentration: 1 nmol/ml, spectral measurement

Protein concentration: 12.5 mg/ml, measured using DC-assay Biorad™

Specific content: 80 pmol/mg protein

**Cytochrome c
Reductase activity:** 3000 nmol/min/mg protein

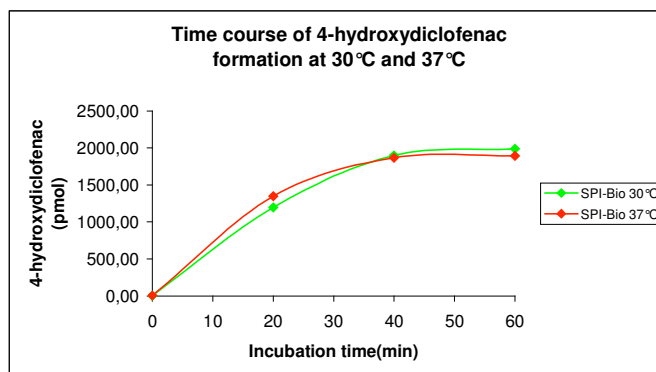
P450 ACTIVITY DATA

Activity measured: diclofenac-4-hydroxylase

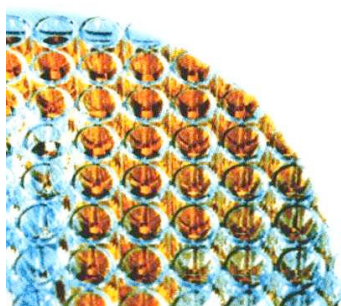
Activity value: 133 pmol/min/pmol P450

For research laboratory
use only.

Not for human
diagnostic use.



Should you wish further information, do not hesitate to contact us.



QC ASSAY METHOD

This assay is specific for the measurement of CYP2C9.

0.2 ml of reaction mixture containing 10 pmol of CYP2C9 is incubated at 30°C for 10 min in 50 mM Tris (pH 7.4), 1 mM EDTA, 600 µM NADPH and 200 µM of diclofenac. Stop reagent: 40µl of acetonitrile/glacial acetic acid (94/6, v/v). 160 µl of acetonitrile are added. Vortex 30 sec and centrifuge 10 min at 10000 rpm, +4°C. Collect supernatant for analysis.

Quantitation is determined in the following HPLC-UV conditions and by using a calibration curve of 4-hydroxy-diclofenac:

Column: Brownlee ODS (5 µm) 2x100 mm ; Temperature: 50°C ; injection volume: 20 µl

Mobile phase: solvent A: H₂O/TFA 0.02% (v/v), solvent B: acetonitrile ; Linear gradient A/B: T0min 85/15 T10min 40/60 following by 0/100 over 2 min ; Flow rate: 1ml/min ; run time: 12 min.

Detection: UV λ = 280 nm

Retention time: 4.6 min (4-hydroxy-diclofenac) – 6.6 min (diclofenac)

N.B.: Time course of 4-hydroxy-diclofenac formation presented in the figure overleaf was obtained in accord with the QC assay method. Other drug/substrate or operating conditions may exhibit different time course of product formation.

ADVICE

Thaw rapidly on ice and keep on ice until use.

Aliquot to minimise freeze-thawing cycles

This assay can be done in a 96-well plate or directly in a tube.

Temperature from 28°C to 37°C may be used.

We strongly suggest to assess your drug/substrate using the buffer mentioned above (assay method).

We suggest to pre-incubate for 5 min. your drug/substrate in the buffer at the temperature you have chosen and start the reaction by adding NADPH.

SAFETY PRECAUTION

The toxicological properties of this reagent have not been investigated. Exercise due care when handling.

Product supplied by SPI-BIO may be harmful if misused. Any product ordered from SPI-BIO must not be used for any purpose other than the intended use specified herein. Please ensure that the product is used safely, and, in particular, that it does not come into direct human contact.

Normal precautions in handling laboratory reagents should be applied. We recommend the use of gloves, lab coats and eye protection when working with any chemical reagents. Do not pipet liquids by mouth. Do not eat, drink or smoke in area in which chemical reagents are handled. Avoid splashing.

FOR FURTHER READING

1. G. Truan, C. Cullin, P. Reisdorf, P. Urban, & D. Pompon. Enhanced in vivo monooxygenase activities of mammalian P450s in engineered yeast cells producing high levels of NADPH-P450 reductase and human cytochrome b5. *Gene* **125**, 49-55 (1993).
2. J.C. Gautier, P. Urban, P. Beaune, & D. Pompon. Engineered yeast cells as model to study coupling between human xenobiotic metabolising enzymes: simulation of the two first steps of benzo[a]pyrene activation. *Eur J Biochem* **211**, 63-72 (1993).
3. P. Urban, G. Truan, & D. Pompon. Xenobiotic metabolism in humanised yeast: engineered yeast cells producing human NADPH-cytochrome P450 reductase, cytochrome b5, epoxide hydrolase and P450s. *Biochem Soc Transac* **21**, 1028-1033 (1993).
4. M.A. Peyronneau, J.P. Renaud, M. Jaouen, P. Urban, C. Cullin, D. Pompon, & D. Mansuy. Expression in yeast of three allelic cDNAs coding for human liver P450 3A4: different stabilities, binding properties and catalytic activities of the yeast-produced enzymes. *Eur J Biochem* **218**, 355-361 (1993).
5. J.P. Renaud, M.A. Peyronneau, P. Urban, G. Truan, C. Cullin, D. Pompon, P. Beaune, & D. Mansuy. Recombinant yeast in drug metabolism. *Toxicology Letters* **82**, 39-52 (1993).
6. D. Pompon, J.C. Gautier, A. Perret, G. Truan and P. Urban. Simulation of human xenobiotic metabolism in microorganisms: yeast a good compromise between E. coli and human cells. *J. Hepatol.* **26** 80-84 (1997).

PURCHASING INFORMATION

By purchasing this product you accept the terms and conditions of supply. Purchasing information is available from SPI-BIO upon request. Material required but not supplied: Buffer, NADPH (or regenerating system), test drug/substrate and distilled or deionized water.