

FT-45078A

Human Cytochrome P450 2C8 (CYP2C8) YR

Description

Catalog number: 45078A, 1 nmol
Name: Human CYP2C8 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*

- stability : 2 years
- **storage temperature : -80 °c (N)**
- Storage buffer: 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- Avoid frequent temperature changes. Thaw on ice.
- Shipping: dry ice
- Materiel required but not supplied: Buffer, NADPH (or regenerating system), test drug/substrate and distilled or deionized water.

Directions for Use

- Thaw rapidly on ice and keep on ice until use.
- Aliquot to minimize 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.

Technical and Scientific Information

Typical batch characteristics

P450 concentration: 1.2 nmol/ml, spectral measurement
Protein concentration: 18.5 mg/ml, measured using Lowry modified protein assay.
Specific content: 65 pmol/mg protein
Cytochrome C Reductase activity: XXXX nmol/min/mg protein

P450 typical Activity Data

Activity measured: diclofenac 4-hydroxylase
Activity value: 6 pmol/min/pmol P450 with human cytochrome b5
 3.3 pmol/min/pmol P450 with human cytochrome b5
 (2 mol cyt b5/1 mol CYP2C8)

Qc Assay Method

- This assay is specific for the measurement of CYP2C8.
- 0.2 ml of reaction mixture containing 10 pmol of CYP 2C8 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: 94 % acetonitrile/ 6 % glacial acetic acid (40 µl). Acetonitrile is added to complete to 200 µl.
- Quantitation of 4-hydroxy-diclofenac is determined in the following HPLC-UV conditions:
 Column: Brownlee ODS (5 µm) 2x100 mm ; Temperature: 50°C ; injection volume: 20µl
 Mobile phase: acetonitrile 15%-H₂O/TFA 85% to acetonitrile 40%-H₂O/TFA 60% over 10 min.; Flow rate: 1ml/min ;
 run time: 12 min.
 Detection: UV λ = 280 nm
 Retention time: 4.6 min (4-hydroxy-diclofenac)

Safety Precaution

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

Product supplied by INTERCHIM may be harmful if misused. Any product ordered from INTERCHIM 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.

Literature

1. [Truan 1993](#) : 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.
2. [Gautier 1993](#) : 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. [Urban 1993](#) : 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. [Peyronneau 1993](#) : 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. [Renaud 1993](#) : 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. [Pompon 1997](#) : 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).

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