

## HUMAN CYTOCHROME P450 3A4 (CYP3A4) HR + Cyt.b5

### ORDERING INFORMATION

**Catalogue Number:** M41113

**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 CYP3A4 and human CYP-reductase coexpressed in *Saccharomyces cerevisiae* mixed with human Cytochrome b5

**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:** 15 mg/ml, measured using DC-assay Biorad™

**Specific content:** 67 pmol/mg protein

**Cytochrome c**

**Reductase activity:** 40 nmol/min/mg protein

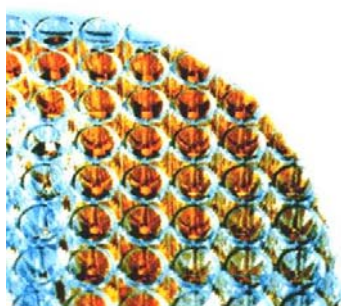
### • P450 ACTIVITY DATA (below typical activity data)

**Activity measured:** Testosterone 6β-hydroxylase

**Activity value:** 13.6 pmol/min/pmol P450 with cytochrome b5

**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 CYP 3A4.
- 0.2 ml of reaction mixture containing 20 pmol of CYP3A4 (cytochrome b5 premixed) is incubated at 30°C for 10 min in 50 mM Tris (pH 7.4), 1 mM EDTA, 200 mM NaCl, 600 µM NADPH and 200 µM of Testosterone. Stop reagent: 1µl of TFA 50 %. 199 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 6β-hydroxy-testosterone:  
Column: Brownlee ODS (5 µm) 2x100 mm ; Temperature: 50°C ; injection volume: 20 µl  
Mobile phase: solvent A: H<sub>2</sub>O/TFA 0.02% (v/v), solvent B: acetonitrile ; Linear gradient A/B: T0min 100/0 T20min 50/50; Flow rate: 1ml/min ; run time: 20 min.  
Detection: UV λ = 254 nm  
Retention time: 8.6 min (6β-hydroxy-testosterone) – 13 min (testosterone)

## • 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. Materiel required but not supplied: Buffer, NADPH (or regenerating system), test drug/substrate and distilled or deionized water.