Human Cytochrome P450 3A4 (CYP3A4) HR

Description

Catalog number: 38043A, 1 nmol
Name: Human CYP3A4 and human 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
- Material 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 nmol/ml, spectral measurement
Protein concentration: 15 mg/ml, measured using Lowry modified protein assay.
Specific content: 67 pmol/mg protein
Cytochrome C Reductase activity: 40 nmol/min/mg protein

P450 typical Activity Data
Activity measured: Testosterone 6β-hydroxylase
Activity value: 13.6 pmol/min/pmol P450 with human cytochrome b5
(2 mol cyt b5/1 mol CYP3A4)

Qc Assay Method
- This assay is specific for the measurement of CYP 3A4.
- 0.2 ml of reaction mixture containing 20 pmol of CYP3A4 (and if required, 40 pmol cytochrome b5) is incubated at 30°C for 10 min in 50 mM Tris (pH 7.4), 1 mM EDTA, 200mM NaCl, 600 µM NADPH and 200 µM of Testosterone. Stop reagent: 1 µl of TFA 50 %. 199 µl of Acetonitrile is added. Centrifuge 10 min at 10000 rpm. Collect supernatant for analysis.
- Quantitation is determined in the following HPLC-UV conditions and by using a calibration curve of 6β-hydroxytestosterone:
  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 100/0 T20min 50/50 ; Flow rate: 1ml/min ; run time: 20 min.
  Detection: UV λ = 254 nm

interbiotech@interchim.com
FT-38043
Retention time: 8.6 min (6β-hydroxy-testosterone) – 13 min (testosterone)

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

Purchasing Information
By purchasing this product you accept the terms and conditions of supply. Purchasing information is available from INTERCHIM upon request.

European Patent No. 0 595 948 - USA Patent No.: 5,635,369 and other V1/April 03

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Not for human diagnostic use.

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