Discovery Life Sciences
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Human CYP3A5 + P450 Reductase + Cytochrome b_5 SUPERSOMES™

Catalog Number….456256
Lot Number.....2402262

Storage Conditions..STORE AT -80°C
Date Released .....2024 March
Expiration Date…….2034 March

Package Contents..…….0.5 nmole cytochrome P450 in 0.5 mL
Protein Content……4.8 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity……....3100 nmole/(min x mg protein)
Cytochrome P450 Content…….....1000 pmole per mL
Cytochrome b_5 Content..…….1200 pmole per mg protein
Testosterone 6β-Hydroxylase Activity…….79 pmole product/(min x pmole P450)

PRODUCT DESCRIPTION: This activity is catalyzed by CYP3A5 which is expressed from human CYP3A5 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase and human cytochrome b_5. A microsome preparation using wild type virus (Catalog No. 456201) should be used as a control for native activities.

ADVICE
- Thaw rapidly in a 37°C water bath. Keep on ice until use.
- Aliquot to minimize freeze-thawing cycles. Minimal loss in catalytic activity was observed after 10 freeze thaw cycles.
- Metabolite production with testosterone is approximately linear for 20 minutes (see graph above). Other substrates may not exhibit similar linearity with respect to incubation time.

HAZARD WARNING:

The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

SAFETY RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

- Do not eat, drink or smoke.
- Avoid contact with skin or eyes.
- Do not inhale aerosols.
- Do not pipette by mouth.
- Wear suitable protective clothing, gloves and eye protection.
- Steam sterilize product or treat product with a 1% solution of sodium hypochlorite prior to disposal.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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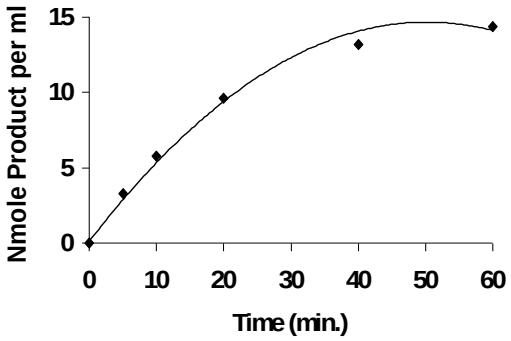
PRIMARY ASSAY METHOD: A 0.50 mL reaction mixture containing 10 pmole P450, 1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 0.2 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 minutes. After incubation, the reaction was stopped by the addition of 250 µL 5 µM 6β hydroxytestosterone-D7 in acetonitrile and centrifuged (10,000 x g) for 3 minutes. The product was detected by LC-MS/MS using its Q1 mass and Q3 mass with positive polarity and quantitated by comparing the peak area ratio to a standard curve of 6β hydroxytestosterone.

Time Course of Product Formation



ANALYTICAL METHOD:

Materials

Column	2.1 x 50 mm 5µm C18 HPLC
Mobile Phase A	0.1% Formic Acid in dH_2O
Mobile Phase B	0.1% Formic Acid in Acetonitrile

Mass Transitions of MRM

Compound	Q1 Mass (amu)	Q3 Mass (amu)
Analyte- 6β -Hydroxytestosterone	305.1 ±0.2	269.1 ±0.2
Internal Standard 6β -Hydroxytestosterone-D7	312.1 ±0.2	276.1 ±0.2

Gradient Separation Conditions

Time (minute)	Flow Composition of Mobile Phase A (%)	Flow Composition of Mobile Phase B (%)	Flow Rate (µL /min)
0.0	75	25	400
1.0	50	50	400
1.2	50	50	400
1.3	10	90	400
1.8	10	90	400
1.9	50	50	400
2.7	50	50	400
2.8	75	25	400
3.5	75	25	400

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21 March 2024

Quality Assurance **Date**

Revision History				
Rev.	**Change No.**	**Description of Change**	**Revised By**	**Revised Date**
1	CC-02544	Updated to Corning branding, protected variable fields, replace safety section with safety statement and clarify SDS [Safety Data Sheet] information, addition of QA signature and date	L. Brown	7/23/14
2	CC-13033	Method section was updated to align with the changes implemented in WB-MP-171 rev. 1. Analytical acquisitions method was converted from text to tables.	C. McCullough	7/24/2019

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