Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798
info@dls.com
https://www.dls.com/

Human CYP1A2 + P450 Reductase SUPERSOMES™

Catalog Number……..456203
Lot Number.....…….2407262

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

Package Contents.....0.5 nmole cytochrome P450 in 0.5 mL

Protein Content.....7.9 mg/mL in 100 mM potassium phosphate (pH 7.4)
Cytochrome c Reductase Activity.....1500 nmole/(min x mg protein)
Cytochrome P450 Content.....1000 pmol per mL
Phenacetin Deethylase Activity.....……34 pmol product/(min x pmol P450)

PRODUCT DESCRIPTION: This activity is catalyzed by CYP1A2 which is expressed from human CYP1A2 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. 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. Less than 20% of the catalytic activity is lost after 6 freeze thaw cycles.
- Western immunoblotting indicates the expressed CYP1A2 has similar mobility as CYP1A2 in human liver microsomes.
- Comparison of Western immunoblotting intensity and spectral P450 contents for this product and human lymphoblast-expressed CYP1A2 indicates that a substantial amount of apoprotein is found in this product.

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 INFORMATION: Safety assessment indicates this product is not hazardous, therefore no SDS (Safety Data Sheet) is provided. Use standard laboratory practices for the handling and disposal of Biosafety Level 1 materials.

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

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PRIMARY ASSAY METHOD: A 0.5 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 phenacetin 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 10 µM acetamidphenol- $^{13}C_2$ ^{15}N in acetonitrile with 0.1% formic acid 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 acetamidophenol.

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-Acetamidophenol	152.0 ±0.2	110.0 ±0.2
Internal Standard-Acetamidophenol- $^{13}C_2$ ^{15}N	155.0 ±0.2	111.0 ±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	98	2	750
0.4	98	2	750
1.0	60	40	750
1.1	2	98	750
1.3	0	100	750
1.4	98	2	750
1.7	98	2	750

Alexa Silies

Quality Assurance

08/29/2024

Date

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