Certificate of Analysis CYP3A5 BACULOSOMES® Plus Reagent, rHuman, 0.5 nmol



Part Number: P2512 Lot Number: 1729869 Immediate Storage: -80°C Shipping Conditions: dry ice 5791 Van Allen Way Carlsbad, CA 92008 Phone: 760.603.7200 Fax: 760.602.6500 www.lifetechnologies.com

Description:

Microsomes prepared from insect cells that were infected with baculovirus containing the cDNAs for human CYP3A5, human cytochrome P450 reductase, and human cytochrome b5.

Protein Content:

12 mg/mL as determined using the Folin-Lowry Protein Procedure.

Cytochrome P450 Content: 1000 pmol/mL.

Specific Content Cytochrome P450: 83.33 pmol/mg of total protein.

Cytochrome b₅ Content: 1900 pmol/mg of total protein.

Cytochrome c Reductase Activity:

5200 nmol of cytochrome c reduced per minute per milligram of protein.

Storage and Handling:

Thaw rapidly in a 37°C water bath. Keep on ice until use. If aliquots are prepared for product storage, volumes less than 50 μ L per aliquot are not recommended. Dilutions of CYP3A5 BACULOSOMES® Plus Reagent, rHuman should be prepared on the day of use, never store diluted. If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase.

A minimal decrease in activity was observed after the microsomes had undergone 10 freeze-thaw cycles.

Storage Buffer:

100 mM potassium phosphate (pH 7.4).

QUALITY ASSURANCE

Lot Specific Testing:

Testosterone 6β-Hydroxylase Activity: 49 pmol product per min per pmol P450.

A 0.5 mL reaction mixture containing 10 pmol CYP3A5, 1.3 mM NADP $^+$, 3.3 mM glucose $^-$ 6-phosphate, 0.4 U/mL glucose $^-$ 6-phosphate dehydrogenase, 3.3 mM MgCl $_2$, and 0.2 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 250 µL acetonitrile and centrifuged (10,000 x g) for 3 minutes. 100 µL of the supernatant was injected into a 4.6 x 250 mm 5 µm C18 HPLC column and separated at 45°C with a mobile phase initially of 58% methanol increasing to 62% methanol over 8 min and at a flow rate of 1.0 mL per min. The product was detected by absorbance at 254 nm and quantitated by comparison to the absorbance of a standard curve for 6 β -hydroxytestosterone.

Nichole Reaksecker

Date: 17/Nov/2015

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