Certificate of Analysis
Vivid® CYP2C9 Red Screening Kit, 1 Kit

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Part Number: P2859
Lot Number: 1680323
Immediate Storage: –80°C
Shipping Conditions: dry ice

Components:

Description	Composition	Quantity	Part Number	Lot Number
CYP2C9 BACULOSOMES® Plus Reagent, rHuman	100 mM Tris (pH 7.5).	0.5 nmol	P2378*	1495735
Vivid® OOMR Substrate	Vivid® OOMR Substrate, dried under vacuum from acetonitrile.	0.1 mg	P2868	1624408A
Vivid® Regeneration System, 100X	333 mM Glucose-6-phosphate and 30 U/mL Glucose-6-phosphate dehydrogenase in 100 mM Potassium Phosphate Buffer (pH 8.0)	0.5 mL	P2878	1670343A
$NADP^+$, 10 mM	$NADP^+$ solution, 10 mM, in 100 mM Potassium Phosphate Buffer (pH 8.0)	0.5 mL	P2879	1508165A
Vivid® CYP450 Reaction Buffer II, 2X	Potassium Phosphate Buffer (100 mM, pH 8.0)	50 mL	P2913	1574691A
Vivid® Red Standard	Lyophilized powder.	0.1 µmol	P2874	1503341A

*See individual COA

Storage and Handling: The performance of this product is guaranteed for 6 months from the date of purchase if stored and handled properly.

Description	Storage and Handling
CYP2C9 BACULOSOMES® Plus Reagent, rHuman	Thaw rapidly in a 37°C water bath. Keep on ice until use. **If aliquots are prepared for product storage, volumes less than 50 µL per aliquot are not recommended.** Dilutions of Vivid® CYP2C9 Red Screening Kit components 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.
Vivid® OOMR Substrate	Store dry substrate at −20°C, and keep protected from light. Once the substrate has been resuspended, store the substrate at −20°C and keep protected from light.
Vivid® Regeneration System, 100X	-80°C
$NADP^+$, 10 mM	-80°C
Vivid® CYP450 Reaction Buffer II, 2X	+4°C or 20-30°C
Vivid® Red Standard	Store at -20°C, desiccated and protected from light.

Certificate of Analysis
CYP2C9 BACULOSOMES® Plus Reagent, rHuman , 0.5 nmol

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Part Number: P2378
Lot Number: 1495735
Immediate Storage: –80°C
Shipping Conditions: dry ice

Description:
Microsomes prepared from insect cells that were infected with baculovirus containing the cDNAs for human CYP2C9, human cytochrome P450 reductase, and human cytochrome b5.

Protein Content:
2.6 mg/mL as determined using the Folin-Lowry Protein Procedure.

Cytochrome P450 Content: 1000 pmol/mL.

Specific Content Cytochrome P450: 385 pmol/mg of total protein.

Cytochrome b_5 Content: 250 pmol/mg of total protein.

Cytochrome c Reductase Activity:
580 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 µL per aliquot are not recommended.** Dilutions of CYP2C9 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.

Less than a 20% decrease in diclofenac 4'-hydroxylase activity was observed after the microsomes had undergone 6 freeze-thaw cycles.

Storage Buffer:
100 mM Tris (pH 7.5).

QUALITY ASSURANCE

Lot Specific Testing:

Diclofenac 4'-Hydroxylase Activity: 29 pmol product per min per pmol P450.

A 0.5 mL reaction mixture containing 10 pmol CYP2C9, 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 diclofenac in 100 mM Tris (pH 7.5) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 100 µL 94% acetonitrile/6% glacial acetic acid and centrifuged (10,000 x g) for 3 minutes. 75 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 20% acetonitrile, 30% methanol with 1 mM perchloric acid in water changing to 100% methanol over 20 min and at a flow rate of 1.0 mL per min. The retention times were approximately 11 min for the 4'–hydroxydiclofenac and 15 min for diclofenac. The product was detected by absorbance at 280 nm and quantitated by comparison to the absorbance of a standard curve for 4'–hydroxydiclofenac.

Becky. Baker, QA Engineer III Date: 27/Mar/2014