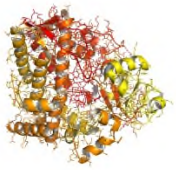
CYP450-GP



PRODUCT NUMBER Hu-P009
HUMAN RCYP4F3b
P450 Enzyme Purified from Sf9 Insect Cells
LOT #3

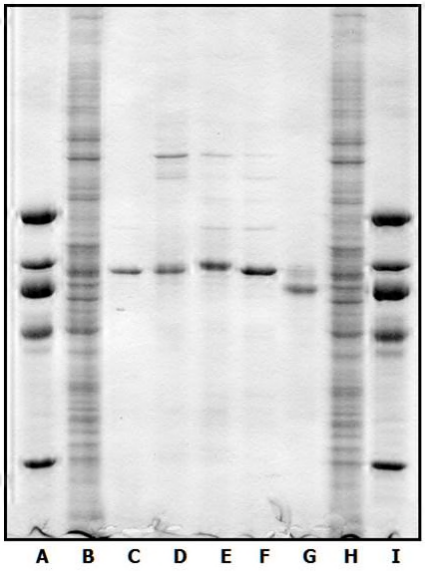
P450 CONTENT =	**8.1 nmol/ml**
PROTEIN CONTENT =	**3.2 mg/ml**
SPECIFIC CONTENT =	**2.5 nmol P450/mg protein**✹

RCYP4F3b was obtained from Sf9 insect cell lysates that had been infected for 72 h with a CYP4F3b cDNA-baculovirus construct in the presence of hemin-bovine serum albumin. The recombinant enzyme was purified using metal-ion affinity chromatography and hydroxylapatite adsorption chromatographies. HumanRCYP4F3b is provided in a solution containing 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.1 mM DTT, and 20% glycerol.

✹The modest P450 specific content of RCYP4F3b stems from the inability of Sf9 cells to fully incorporate heme from the hemin-BSA complex in the culture media into the expressedRCYP4F3b apoprotein.

♦ Purity

Purity has been determined by electrophoresis on 7.5% acrylamide gels run with the discontinuous buffer system. RCYP4F3b migrates as a single band with a molecular weight of 57.0 kDa (see Fig. 1, lane D), and is a low-spin hemeprotein when oxidized with a ferrous carbonyl Soret maximum at 451 nm.



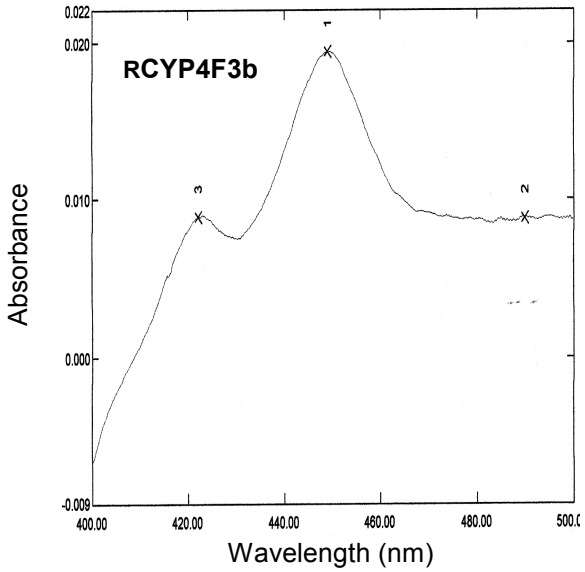
PAGE analysis of purified human recombinant CYP4F/A enzymes
Lanes A & I, Molecular Weight Standards (68 kDa, 58 kDa, 53 kDa, 43 kDa and 29 kDa, top to bottom, 1 µg each)
Lane B & H, Lysates from CYP4F-transfected Sf9 Cells (20 µg)
Lane C, Purified RCYP4F2 (1.5 µg)
Lane D, **Purified RCYP4F3b** (1.5 µg)
Lane E, Purified RCYP4F11 (1.5 µg)
Lane F, Purified RCYP4F12 (1.5 µg)
Lane G, Purified RCYP4A11 (1.5 µg)

♦ Reconstitution

RCYP4F3b catalytic activity is assessed upon reconstitution of the enzyme with human liver NADPH:P450 reductase, synthetic dilauroyl-phosphatidlycholine and cytochrome b_5. Full details for reconstitution and metabolism are given below.

♦ **Storage** RCYP4F3b should be stored @ -80°C. Avoid repeated freeze-thawing cycles.

CO Reduced Difference Spectrum of PurifiedRCYP4F3b



FATTY ACID, LEUKOTRIENE B_4 AND γ–TOCOPHEROL OXIDATION BY RECOMBINANT HUMAN CYP4F AND CYP4A11 P450 ENZYMES

	SUBSTRATE					
ENZYME	AA	LAURATE	3-OH PALMITATE	OLEATE	LTB4✺	γ-TOC✺
Human Liver Mx	2.0 ± 0.9 (4)	9.7 ± 2.7 (10)	9.5	7.0 ± 0.4 (7)	683.5 ± 112 (8)	29.9
RCYP4F2	2.5 ± 0.9 (6)	< 0.1	3.7	20.8	217.8	< 0.1
RCYP4F3b	**0.8**	**< 0.1**	**2.9**	**1.6**	**216.9**	**< 0.1**
RCYP4F11	2.03	7.1 ± 3.2 (3)	35.0	7.7	185.8	119.2
RCYP4F12	< 0.1	nd	< 0.1	< 0.1	< 0.1	< 0.1
RCYP4A11	1.6	41.2	1.7	2.6	nd	nd

Oxidation of arachidonate, laurate, oleate, 3-hydroxypalmitate, LTB_4, and γ–TOC to their ω–hydroxylated metabolites was performed in reaction mixtures (0.25 ml) containing: a) purified reconstituted systems consisting of 25 pmol P450 enzyme, 75 pmol P450 reductase, 7.5 µg dilauroylphosphatidylcholine and 100 pmol b_5 or; b) human liver microsomes (protein equivalent to 50-150 pmol P450). Other incubation components included 100 mM potassium phosphate buffer (pH 7.4), 1 mM NADPH, and the above-mentioned substrates at a final concentration of 100 µM except in the case of LTB4 (30 µM). All reactions were initiated with NADPH, and were terminated after 10-15 min at 37°C. Formation of 20-HETE, 12-hydroxylaurate, 3,16-dihydroxypalmitate, 18-hydroxyoleate, 20-hydroxy LTB4 and 13-OH γ–TOC were then determined as described elsewhere.

Product formation is expressed as nmol metabolite formed/min/nmol P450, and represents the average of either two different experiments or the mean ± SD (# of experiments in parentheses).
✺Results are expressed as pmol product formed/min/nmol P450