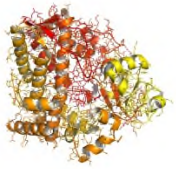
V2; 12/01/19

CYP450-GP



PRODUCT NUMBER Hu-P012
HUMAN RCYP4F12
P450 Enzyme Purified from Sf9 Insect Cells
LOT #1

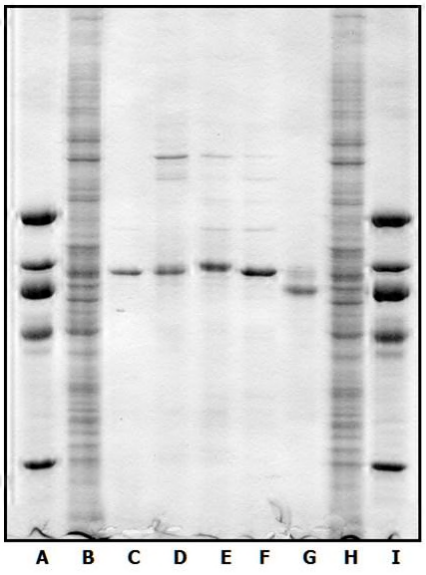
P450 CONTENT =	**18.0 nmol/ml**
PROTEIN CONTENT =	**3.2 mg/ml**
SPECIFIC CONTENT =	**5.6 nmol P450/mg protein**

RCYP4F12 was obtained from Sf9 insect cell lysates that were infected for 72 h with a CYP4F12 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. Human RCYP4F12 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 low P450 specific content of RCYP4F12 stems from the inability of Sf9 cells to fully incorporate heme from the hemin-BSA complex in culture media into the expressed RCYP4F12 apoprotein.

♦ Purity

Purity has been determined by electrophoresis on 7.5% acrylamide gels run with the discontinuous buffer system. RCYP4F12 migrates as a single band with a molecular weight of 56.5 kDa (see Fig. 1, lane F), 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 (1.0 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

RCYP4F12 catalytic activity is assessed upon reconstitution of the enzyme with NADPH:P450 reductase, synthetic dilauroylphosphatidlycholine and cytochrome b_5. Full details for reconstitution and metabolism are given below. .

♦ **Storage** RCYP4F12 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.

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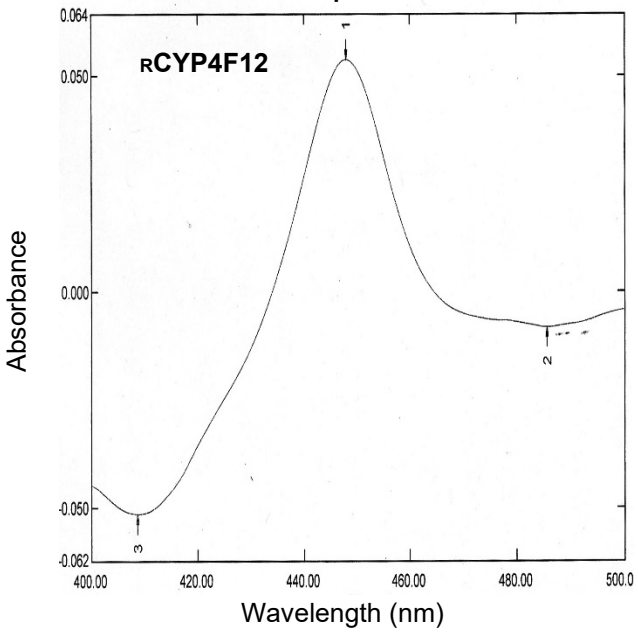
CO Reduced Difference Spectrum of Purified RCYP4F12



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

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