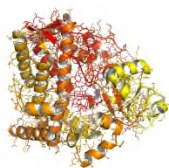


# CYP450-GP



**PRODUCT NUMBER Hu-P011**  
**HUMAN RCYP4F11**  
 P450 Enzyme Purified from Sf9 Insect Cells  
**LOT #1**

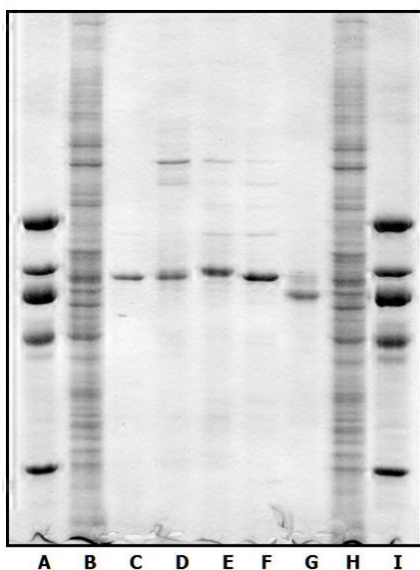
P450 CONTENT = **9.9 nmol/ml**  
 PROTEIN CONTENT = **2.3 mg/ml**  
 SPECIFIC CONTENT = **4.3 nmol P450/mg protein**

RCYP4F11 was derived from lysates derived from Sf9 insect cells that were infected for 72 h with a CYP4F11 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 RCYP4F11 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 RCYP4F11 stems from the inability of Sf9 cells to fully incorporate heme from the hemin-BSA complex in culture media into the expressed RCYP4F11b apoprotein.

## ◆ Purity

Purity has been determined by electrophoresis on 7.5% acrylamide gels run with the discontinuous buffer system. RCYP4F11 migrates as a single band with a molecular weight of 58.0 kDa (see Fig. 1, lane C), and is a low-spin heme protein when oxidized with a ferrous carbonyl Soret maximum at 451 nm.



## PAGE analysis of purified human recombinant CYP4F/A enzymes

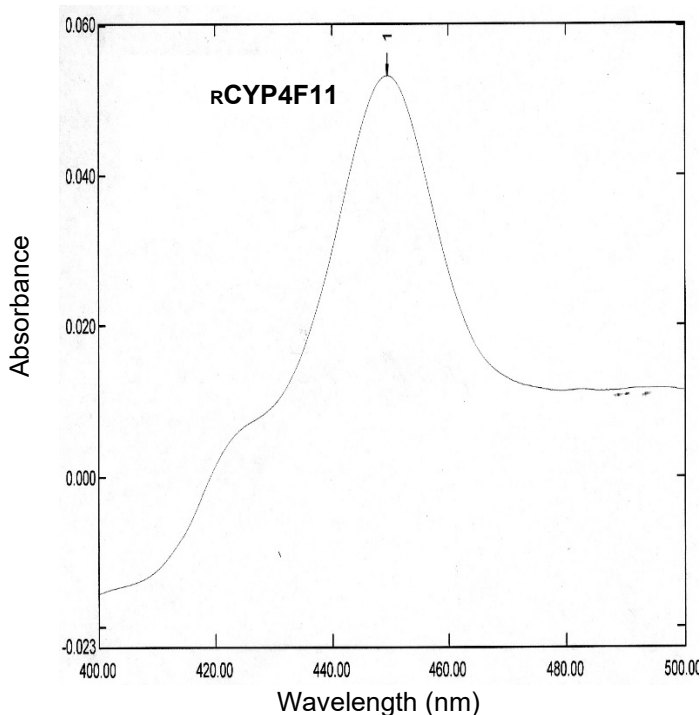
Lanes A & I, Molecular Weight Standards (1.0 µg each)  
 Lane B & H, Lysates from CYP4F-transfected Sf9 Cells (20 µg)  
 Lane C, Purified RCYP4F2 (1.5 µg)  
 Lane D, Purified RCYP4F3b (1.5 µg)  
**Lane E, Purified RCYP4F11 (1.5 µg)**  
 Lane F, Purified RCYP4F12 (1.5 µg)  
 Lane G, Purified RCYP4A11 (1.5 µg)

## ◆ Reconstitution

RCYP4F11 catalytic activity is assessed upon reconstitution of the enzyme with NADPH:P450 reductase, synthetic dilauroylphosphatidylcholine and cytochrome b<sub>5</sub>. Full details for reconstitution and metabolism are given below.

◆ **Storage** RCYP4F11 should be stored @ -80°C. Avoid repeated freeze-thawing cycles.

## CO Reduced Difference Spectrum of Purified RCYP4F11

FATTY ACID, LEUKOTRIENE B<sub>4</sub> AND  $\gamma$ -TOCOPHEROL OXIDATION BY RECOMBINANT HUMAN CYP4F AND CYP4A11 P450 ENZYMES

ENZYME	SUBSTRATE					
	AA	LAURATE	3-OH PALMITATE	OLEATE	LTB <sub>4</sub> *	$\gamma$ -TOC*
Human Liver Mx	2.0 $\pm$ 0.9 (4)	9.7 $\pm$ 2.7 (10)	9.5	7.0 $\pm$ 0.4 (7)	683.5 $\pm$ 112 (8)	29.9
RCYP4F2	2.5 $\pm$ 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
<b>RCYP4F11</b>	<b>2.03</b>	<b>7.1 <math>\pm</math> 3.2 (3)</b>	<b>35.0</b>	<b>7.7</b>	<b>185.8</b>	<b>119.2</b>
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<sub>4</sub>, and  $\gamma$ -TOC to their  $\omega$ -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  $\mu$ g dilauroylphosphatidylcholine and 100 pmol b<sub>5</sub> 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  $\mu$ M except in the case of LTB<sub>4</sub> (30  $\mu$ M). 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 LTB<sub>4</sub> and 13-OH  $\gamma$ -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  $\pm$  SD (# of experiments in parentheses).

\*Results are expressed as pmol product formed/min/nmol P450