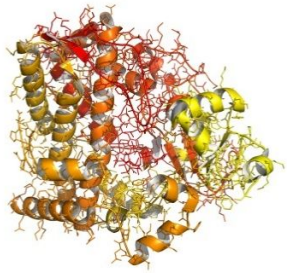


CYP450-GP
2870 Scott Street
Suite 103
Vista, CA 92081
Phone 760.295.7426
Fax 760.418.6472
support@cyp450-gp.com

P450 ENZYME IMMUNOQUANTITATION

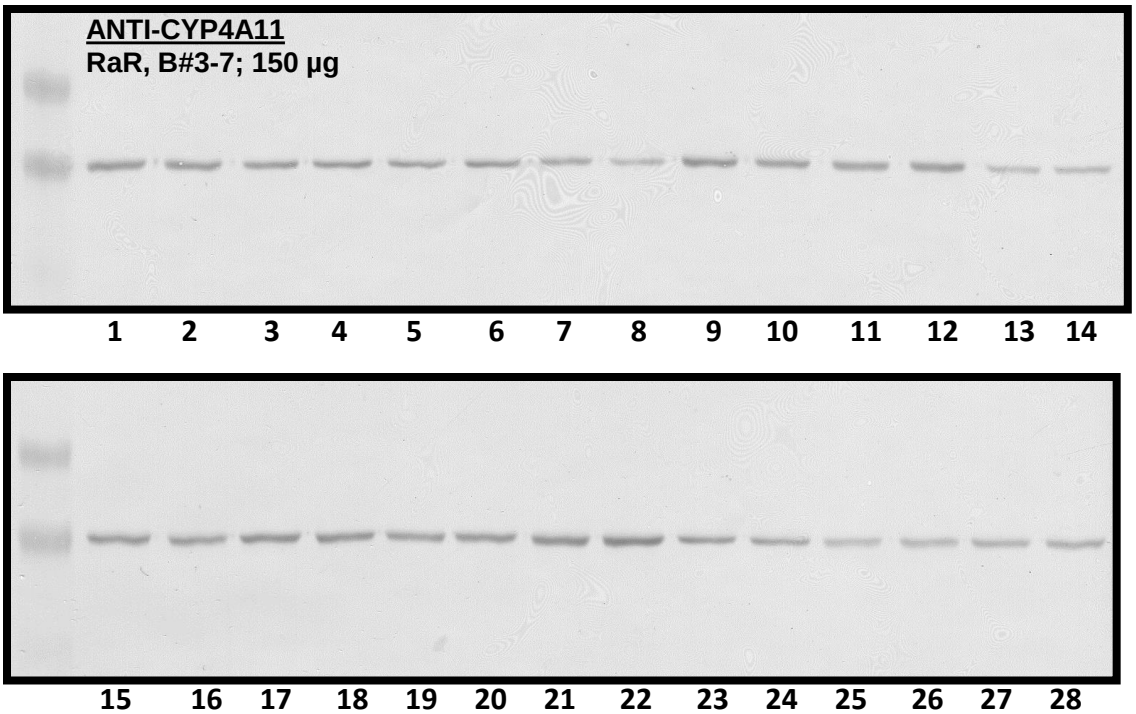
Knowledge of the P450 enzyme composition in microsomes derived from liver or extrahepatic tissues is highly valuable when interpreting the results of *in vitro* NME metabolism studies. Such information is used to perform correlation analyses whereby rates of oxidative metabolism of a given compound by liver microsomes derived from multiple donors are correlated with immunoreactive CYP450 enzyme levels in the same sample. This approach has proved rather fruitful because individual CYP450 enzyme levels not only vary markedly among subjects (up to 100-fold) but also differ independently from each other.

CYP450-GP utilizes protein or "Western blotting" to quantitate the amounts of specific P450 enzymes in microsomes from human tissues. We have had a long history of using this powerful technique for measuring the hepatic and renal levels of P450s involved in both xenobiotic and endobiotic metabolism (see *LITERATURE*). Western blotting is also an integral method for antibody characterization at our facility. Successful Western blotting for P450 enzyme immunoquantitation depends primarily on the quality of the primary (1°) antibody utilized. At CYP450-GP, we take pride in knowing that our rabbit anti-human P450 primary antibodies are among the finest available anywhere, and can provide highly consistent and reproducible results.

The methodology we use to quantitate either one or multiple P450 proteins in samples of liver (or extrahepatic) microsomes is given in our Western Blotting procedure (see PROTOCOLS). The test sample is applied in different amounts to the original SDS-PAGE gel which also contains a reference sample of liver microsomes with previously-established P450 enzyme levels. After completion of the electrophoretic procedures, the blots are immunochemically stained with the P450 antibody of interest. Comparing the P450 staining intensities of the unknown liver sample to those of the previously characterized sample allows for assignment in the former of relative P450 enzyme levels.

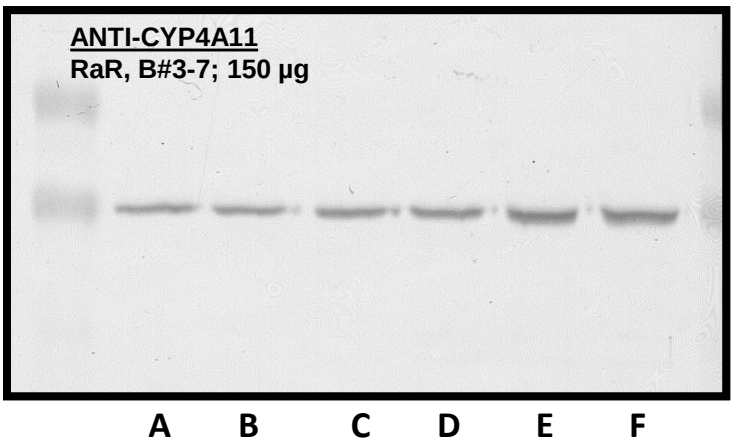
Please contact sales@cyp450-gp.com to learn more about our P450 Enzyme Immunoquatitation offerings.

IMMUNOQUANTITATION OF CYP4A11 CONTENT
IN HUMAN LIVER MICROSOMES



LANE	SAMPLE	P450	CYP4A11	
		nmol/mg✚	Protein■	Activity✷
1,2	UC9410 Mx	0.167	78.8	0.76
3,4	UP879 Mx	0.205	42.7	2.24
5,6	UP1091 Mx	0.159	41.7	0.97
7,8	UC8932 Mx	0.150	33.4	0.41
9,10	UC8928 Mx	0.163	35.2	1.34
11,12	UC9001 Mx	0.304	58.4	1.30
13,14	UC8926 Mx	0.238	9.4	0.60
15,16	UC9410 Mx	0.167	78.8	0.76
17,18	UP1065 Mx	0.217	21.0	1.63
19,20	UP1095 Mx	0.276	31.5	1.29
21,22	UC8912 Mx	0.195	54.7	1.85
23,24	UN505 Mx	0.213	26.0	0.65
25,26	UC9409 Mx	0.320	52.9	3.46
27,28	UP1072 Mx	0.181	31.8	1.11

All samples were analyzed in duplicate, and the values given denote the average
✚P450 values (nmol/mg protein) denote the aggregate P450 content determined spectrophotometrically
■CYP4A11 values are expressed as pmol /mg protein.
✷Laurate hydroxylase activity is expressed as nmol 12-OH laurate formed/min/mg protein.



LANE	SAMPLE	AMOUNT
A	UC9410	7.5 µg
B	UC9410	7.5 µg
C	UC9410	10.0 µg
D	UC9410	10.0 µg
E	UC9410	15.0 µg
F	UC9410	15.0 µg