

#### Technical tip

Xenobiotic biotransformation is the principal mechanism for maintaining homeostasis during exposure of organism to foreign molecules such as drugs. It is accomplished by limited number of enzyme with broad substrate specificities. Reactions catalyzed by xenobiotic-biotransforming enzyme are divided into two groups, called phase I and phase II, resulting in an increase in xenobiotic hydrophilicity, enhancing greatly their elimination.

Among the phase I biotransforming enzymes, Cytochrome (CYP) P450s ranks first in terms of oxydation catalytic versatility and the broad number of xenobiotics they detoxify or activate to reactive intermediates, that may or may not been taken in charge by phase II enzymes.

The highest concentration of P450 enzymes involved in xenobiotic biotransformation is found in liver endoplasmic reticulum (microsomes) but some cytochrome P450s are also present in most other tissues.

Being the major elimination pathway for many drugs, P450 enzymes play a very important role in the intensity and duration of drugs, in the detoxication of xenobiotics, but may also lead to toxic or tumorigenic metabolites.

All P450 enzymes are heme-containing proteins. The heme iron is usually ferric (Fe<sup>3+</sup>). Once reduced to the ferrous (Fe<sup>2+</sup>) state, it allows CYP P450s to bind O<sub>2</sub> and carbon monoxide (CO). The complex between ferrous cytochrome and CO absorbs light at 450 nM (max). This explains derives they name CYP P450s.

Liver microsomal P450 mainly belong to four gene families (CYP1, CYP2, CYP3 and CYP4). These gene families generally include to a single subfamily (CYP 1A, CYP 3A or CYP 4A), excepted the CYP 2 gene family which contains five subfamillites (CYP 2A, CYP 2B, CYP 2C, CYP 2D and CYP 2E). The number of CYP 450 enzymes in each subfamily differs from one species to another.

Literature : Szczebara FM et al., 2003 - Total biosynthesis of hydrocortisone from a simple carbon source in yeast, Nat. Biotechnol. 2003 Feb;21(2):143-9.

See below for detailed information.

Interchim proposes you a range of main P450 enzymes contained in human liver microsomes i.e. :

Description	Cat.#	Qty
Human Cytochrome P450 1A1 (CYP1A1) YR	38600A	1 nmol
Human Cytochrome P450 1A2 (CYP1A2) YR	38041A	1 nmol
Human Cytochrome P450 3A4 (CYP3A4) HR	38043A	1 nmol
Human Cytochrome P450 3A5 (CYP3A5) HR	39187A	1 nmol
Human Cytochrome P450 2A6 (CYP2A6) YR	30828A	1 nmol
Human Cytochrome P450 1B1 (CYP1B1) YR	85203A	1 nmol
Human Cytochrome P450 2D6 (CYP2D6) YR	38851A	1 nmol
Human Cytochrome P450 2E1 (CYP2E1) YR	38042A	1nmol
Human Cytochrome P450 Control HR (Human Red)	AK8030	10 ng
Human Cytochrome P450 Control YR (Yeast Red)	AK8040	10 ng
Pooled human liver microsomes	732014	50 mg

#### Human Cytochrome P450 1A1 (CYP1A1) YR

- ◆ Stability : at least 2 years at -80°C
- ◆ Contents : Human CYP1A1 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (1 nmol/ml), Protein concentration (9.5 mg/ml), Specific content (105 pmol/mg protein), Cytochrome C Reductase Activity (1200 nmol/min/mg protein)
- ◆ QC assay method : 7-ethoxyresorufin-O-deethylase
- ◆ Turn-over : 30 min<sup>-1</sup>

#### Human Cytochrome P450 1A2 (CYP1A2) YR - 38041A

- ◆ Stability : at least 2 years at -80°C
- ◆ Contents : Human CYP1A2 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (1 nmol/ml), Protein concentration (52 mg/ml), Specific content (19 pmol/mg protein), Cytochrome C Reductase Activity (1100 nmol/min/mg protein)
- ◆ QC assay method : 7-ethoxyresorufin-O-deethylase
- ◆ Turn-over : 2.4 min<sup>-1</sup>

### Human Cytochrome P450 3A4 (CYP3A4) HR - 38043A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP3A4 and human CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (1 nmol/ml), Protein concentration (15 mg/ml), specific content (67 pmol/mg protein), Cytochrome C Reductase Activity (40 nmol/min/mg protein)
- ◆ QC assay method : Testosterone 6β-hydroxylase
- ◆ Turn-over : 13.6 min<sup>-1</sup> (+b5)

### Human Cytochrome P450 3A5 (CYP3A5) HR - 39187A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP3A5 and human CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (1 nmol/ml), Protein concentration (26 mg/ml), Specific content (39 pmol/mg protein), Cytochrome C Reductase Activity (96 nmol/min/mg protein)
- ◆ QC assay method : 13Testosterone 6α-hydroxylase
- ◆ Turn-over : 3 min<sup>-1</sup> (+b5)

### Human Cytochrome P450 2A6 (CYP2A6) YR - 30828A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP2A6 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ QC assay method : coumarin-7-hydroxylase

### Human Cytochrome P450 1B1 (CYP1B1) YR - 85203A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP1B1 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (0.5 nmol/ml), Protein concentration (85 mg/ml), Specific content (6 pmol/mg protein), Cytochrome C Reductase Activity (960 nmol/min/mg protein)
- ◆ QC assay method : 7-ethoxyresorufin-O-deethylase
- ◆ Turn-over : 3.1 min<sup>-1</sup>

### Human Cytochrome P450 2D6 (CYP2D6) YR - 38851A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP2D6 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (0.7 nmol/ml), Protein concentration (101 mg/ml), Specific content (7 pmol/mg protein), Cytochrome C Reductase Activity (1293 nmol/min/mg protein)
- ◆ QC assay method : 5.4 dextromethorphan-O-demethylase
- ◆ Turn-over : 96 min<sup>-1</sup>

### Human Cytochrome P450 2E1 (CYP2E1) YR - 38042A

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human CYP2E1 and yeast CYP-reductase coexpressed in *Saccharomyces cerevisiae*
- ◆ Storage buffer : 50 mM Tris (pH 7.4), 1 mM EDTA, 20 % glycerol
- ◆ Typical data : P450 concentration (1 nmol/ml), Protein concentration (15 mg/ml), Specific content (65 pmol/mg protein), Cytochrome C Reductase Activity (1335 nmol/min/mg protein)
- ◆ QC assay method : p-nitrophenol hydroxylase
- ◆ Turn-over : 14 min<sup>-1</sup>

### Human Cytochrome P450 Control HR (Human Red) - AK8030

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human microsomes from a cell line containing an empty cDNA insert isolated from *Saccharomyces cerevisiae* host coexpressed with human reductase
- ◆ Typical data : Protein concentration (10 mg/ml), Cytochrome C Reductase Activity (61 nmol/min/mg protein)

## Technical tip

### ADMETOX

Administration, Metabolism and Toxicology studies are performed in vitro at several steps of drug discovery to avoid wasting time developing libraries based on unfavourable chemical structures to that point.

At the earliest steps, P450 assays may be included to discard those compounds metabolized by 2D6 P450 isoforms when it is known the isoform generate many drug:drug interactions, or at the opposite to select proDrugs that should be metabolized by definite P450 isotypes in some organs prior to be active.

Elsewhere, P450 assays may be preferred at an intermediate step of drug discovery to screen large libraries to maximize drug bio-availability or minimize toxicology risks. P450 may be again needed in final steps of drug selection to prepare big amount of drug metabolite that should further be assayed for pharmac-activity, -kinetics or also -toxicology.

Hepatocytes are a versatile and popular approach to test metabolic stability, in vitro toxicity but also species differences. Several applications and limitations were addressed using isolated hepatocytes, fresh, cryopreserved or cultured (useful for enzyme induction).

*Now, pooled isolated microsomes are designed primarily for P450 inhibition testing, and reaction phenotyping.*

P450 isotypes and recombinant P450 systems especially allow enzyme mapping.

# Drug Discovery : HTS screening & toxicology

## Microsomes P450

### Human Cytochrome P450 Control YR (Yeast Red) - AK8040

- ◆ Stability : ~ 2 years at -80°C
- ◆ Contents : Human microsomes from a cell line containing an empty cDNA insert isolated from *Saccharomyces cerevisiae* host coexpressed with yeast reductas
- ◆ Typical data : Protein concentration (10 mg/ml), Cytochrome C Reductase Activity (554 nmol/min/mg protein)

### Pooled Human Liver Microsomes

Endoplasmic reticulum that carries cytochromes P-450 for drug characterisation

- ◆ Prepared from homogenates of fresh human liver
- ◆ Pooled from at least 15 livers (graft surplus : initial material with clinical grade)
- ◆ Evaluable in large quantities

*Controls* : protein concentration (20-40 mg proteins/ml), cytochrome P-450 total content, enzyme activities (phenacetin deethylase, coumarin hydroxylase, tolbutamide hydroxylase, S-mephenytoin hydroxylase, dextromethorphan demethylase, chlorzoxazone hydroxylase and nifedipine oxidase activities)

Description	Cat.#	Qty
Pooled human liver microsomes	732014	50 mg

Please inquiry for Bulk quantity

Description	Cat.#	Qty
AAMU	U10555	5 mg
ACETOMINOPHEN (Paracetamol)	U10715	250 mg
AHMC, AMMC metabolite standard	820421	5 mg
2-AMINO-5-CHLOROBENZOXAZOLE	U10700	5 mg
1-AMINOBENZOTRIAZOLE	408750	5 mg
AMMC, FLUOR. 2D6 SUBSTRATE	820412	5 mg
4-ANDROSTENE-3,17-DIONE	U10400	5 mg
ARACHIDONIC ACID, Na salt	U10685	5 mg
BENZDAMINE N-OXIDE H.MAL. Salt	U10560	5 mg
1-BENZYLIMIDAZOLE	04078K	5 g
7-BENZYLOXYGUINOLINE	U10616	5 mg
7-BENZYLOXYRESORUFIN	47569A	10 mg
7-BENZYLOXY-4-TFC	799453	5 mg
BERGAMOTIN	512355	5 mg
BUFURALOL, HCl salt	850195	10 mg
CARBOXYTOLBUTAMIDE	975151	10 mg
CARBOXYTOLBUTAMIDE ME ESTER	U06860	5 mg
CHLORZOXAZONE	092540	5 mg
CLOFIBRATE	114150	5 mg
COUMARIN	008480	5 mg
3-CYANO-7-ETHOXYCOUMARIN	57543A	10 mg
3-CYANO-7-METHOXYCOUMARIN	U10590	5 mg
DAPSONE	U10600	5 mg
DEBRISOQUIN SULPHATE	U10280	5 mg
DESETHYLOXYBUTYNIN HCl salt	U08255	5 mg
0-DESMETHYL NAPROXEN	U10440	5 mg
0-DESMETHYL TRAMADOL	U10430	5 mg
DESMETHYLSELEGILENE	U08020	5 mg
DEXTROMETHORPHAN HYDROBROMIDE	U10490	5 mg
DEXTORPHAN	854900	5 mg
DICLOFENAC	336676	5 g
6',7'-DIHYDROXYBERGAMOTIN	R47950	5 mg
5,5-DIPHENYLHYDANTOIN	061000	5 mg
ESTRONE-3-GLUCURONIDE, Na salt	U10475	2 mg
7-ETHOXYRESORUFIN	40238A	5 mg
7-ETHOXY-4-TRIFLUOROMETHYLCOUMARIN	M1221A	25 mg
FURAFYLLINE	310680	5 mg
HALOPERIDOL	358870	5 mg
HALOPERIDOL, reduced	396790	5 mg
HYDROCORTISONE	130170	5 mg
HYDROMORPHONE-3-GLUCURONIDE	U10240	5 mg
HYDROXYBUFURALOL MALEATE	666741	5 mg
6-HYDROXYCHLORZOXAZONE	588550	5 mg
6-BETA-HYDROXYCORTISOL	U10530	5 mg
7-HYDROXYCOUMARIN GLUCURONIDE	U10270	5 mg
7-HYDROXYCOUMARIN POTASSIUM SULFATE	G76991	5 mg
DL-4-HYDROXYDEBRISOQUIN SULPHATE	982120	5 mg
4'-HYDROXYDICLOFENAC	413043	50 nmol

Description	Cat.#	Qty
4'-HYDROXYDICLOFENAC	413041	5 mg
3-HYDROXYGUINIDINE	U10420	5 mg
7-HYDROXYGUINOLINE	T38670	5 mg
12-HYDROXYLAURIC ACID	U10520	5 mg
4'-HYDROXYMEPHENYTOIN	489921	5 mg
1'-HYDROXYMIDAZOLAM	534310	5 mg
4-HYDROXYMIDAZOLAM	534320	5 mg
+3-HYDROXYMORPHINAN	929240	5 mg
4'-HYDROXYNIRVANOL	975142	5 mg
16-ALPHA-HYDROXYTESTOSTERONE	U10340	5 mg
2-ALPHA-HYDROXYTESTOSTERONE	U10300	5 mg
6-ALPHA-HYDROXYTESTOSTERONE	U10320	5 mg
7-ALPHA-HYDROXYTESTOSTERONE	U10330	5 mg
16-BETA-HYDROXYTESTOSTERONE	U10350	5 mg
6-BETA-HYDROXYTESTOSTERONE	U10290	5 mg
2-BETA-HYDROXYTESTOSTERONE	U10310	5 mg
11-ALPHA-HYDROXYTESTOSTERONE	U10740	5 mg
7-HYDROXY-4-TFMC-SULPHATE ET3N SALT	U10690	5 mg
4-HYDROXYTOLBUTAMIDE	49010A	10 mg
7-HYDROXY-4-TRIFLUOROMETHYLCOU	U10395	100 mg
4'-HYDROXYWARFARIN	596981	5 mg
10-HYDROXYWARFARIN	597241	5 mg
6-HYDROXYWARFARIN	596971	5 mg
8-HYDROXYWARFARIN	596991	5 mg
7-HYDROXYWARFARIN	597041	5 mg
ITRACONAZOLE	A28159	50 mg
KETOCONAZOLE	431108	500 mg
LAURIC ACID	135150	5 mg
(S)-(+)-MEPHENYTOIN	704647	10 mg
(R)-(-)-MEPHENYTOIN	20560A	5 mg
MEPHENYTOIN	413120	5 mg
METHOXSALEN	U10640	5 mg
4'-METHOXYMEPHENYTOIN	190460	5 mg
3-METHOXYMORPHINAN HYDROCHLORIDE	990471	5 mg
7-METHOXYRESORUFIN	U10460	5 mg
METYRAPONE	U10650	5 mg
MIDAZOLAM HYDROCHLORIDE	534230	5 mg
MOCLOBEMIDE	E59695	10 mg
MONOACETYL DAPSONE	U10540	5 mg
MORPHINE-3,6-GLUCURONIDE	U07730	5 mg
MORPHINE-3-GLUCURONIDE	U07980	5 mg
MUSCONE	S02631	100 mg
NALOXONE-3-GLUCURONIDE	U10450	5 mg
NIFEDIPINE (CYP3A4 Substrat)	227759	1 g
NIFEDIPINE, oxidized	137300	5 mg
(R)-(-)-NIRVANOL	205610	5 mg
(S)-(+)-NIRVANOL	205628	25 mg
DL-NIRVANOL	067181	5 mg
OMEPRAZOLE	R68088	100 mg
9-(3-OXOPROP-1 -ENYL)ADENOSINE	U10260	5 mg
PACLITAXEL (TAXOL)	08426A	5 mg
PARACETAMOL SULPHATE	U10570	5 mg
7-PENTOXYRESORUFIN	886280	5 mg
PHENACETIN	HE2050	250 mg
PHENYL-D5-7-HYDROXYWARFARIN	U10760	5 mg
D5-PHENYTOIN	U10770	5 mg
PROADIFEN (SKF 525A)	U10370	5 mg
PROGESTERON	U10670	5 mg
PROGESTERONE-11A-GLUCURONIDE	U08010	5 mg
QUERCETIN	E77318	100 mg
QUINIDINE	U10380	5 mg
RESORUFIN	954328	100 mg
SALICYL GLUCURONIDE	U10510	5 mg
SALICYL SULPHATE, Na salt	U10410	5 mg
SODIUM DIETHYLDITHIOCARBAMATE	U10500	5 mg
SULPHAPHENAZOLE	859430	5 mg
TERFENADINE ALCOHOL METABOLITE	U10250	5 mg
TERFENADINE CARBOXYLATE	U09580	5 mg
TESTOSTERONE	053400	5 mg
TOLBUTAMIDE	297890	5 mg
TRAMADOL	A16360	5 mg
TRANLYCYPROMIN	U10730	5 mg
TROLEANDOMYCIN	662750	5 mg
(R)-(+)-WARFARIN	968811	100 mg
S-(-)-WARFARIN	968781	100 mg
WARFARIN, Na salt	597471	5 mg