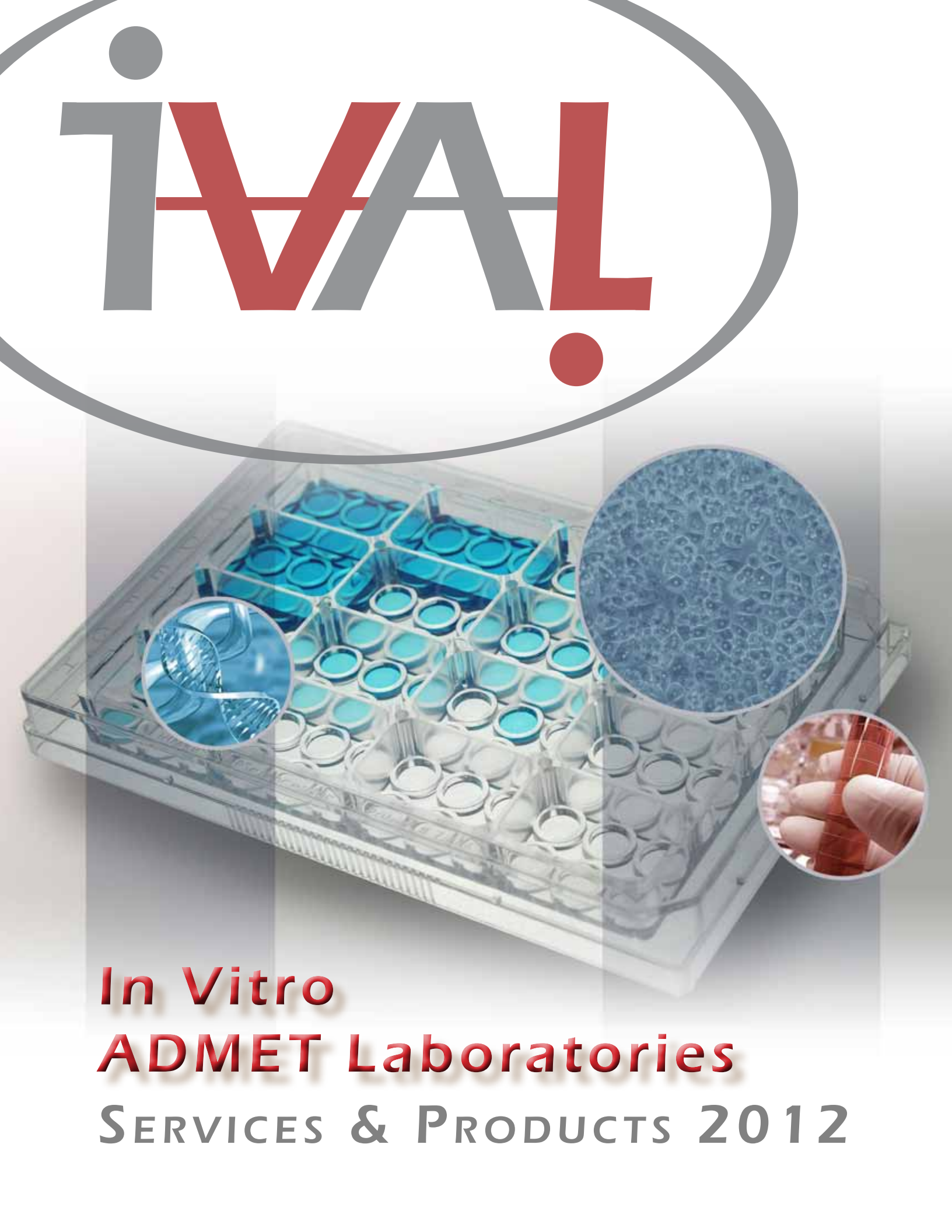


In Vitro ADMET Laboratories

Services & Products 2012

Dear Colleague:

IVAL is proud to present our products and contract services to you that represent our past three decades of expertise in the application of in vitro experimental systems to evaluate drug absorption, metabolism, drug-drug interactions and drug toxicity.

Besides the “routine” in vitro ADME studies that are also offered by other contract research organizations, we have developed higher throughput screening assays to aid the selection of best drug candidates for during early phases of drug development: Organ-specific toxicity evaluation (e.g. hepatotoxicity, nephrotoxicity, and neurotoxicity) using primary cells either as mono-cell type studies or as multiple organ co-cultures using our patented Integrated Discrete Multiple Organ Co-culture (IdMOC™) experimental system; human hepatocyte P450 inhibition assays, and human hepatocyte P450 induction assays; as well as a comprehensive screening assay for adverse drug properties.

I will appreciate receiving feedback from you on our service offerings and our PhD scientists will be more than happy to provide expert consultations and assistance to your technical questions. Please feel free to contact me by email at: lialbert@invitroadmet.com.

With my best regards,

Albert P. Li, Ph. D.
President and CEO
In Vitro ADMET Laboratories

IVAL

In Vitro Toxicity Screening Services

- Multiple Organ Toxicity Screening
- Organ-specific Toxicity Screening
- Anti-cancer Drug Screening

In Vitro Drug Metabolism and Toxicity Screening Services

- Simultaneous Screening for In Vitro Drug Metabolism and Toxicity
- Primary Cell Cytotoxicity Assay
- Drug-drug Interaction Assay
- Pathway Determination Assay
- P450 Inhibition Assay
- P450 Induction Assay
- Gene Expression Assay
- High-throughput Adverse Drug Effect Assay (HTS-ADE)
- ADME Assay
  - *Metabolite Profiling*
  - *Metabolic Stability*

In Vitro Research Products

- IdMOC™ Plates
- Cell Culture Media
- Collagen Coated Vessels

Services

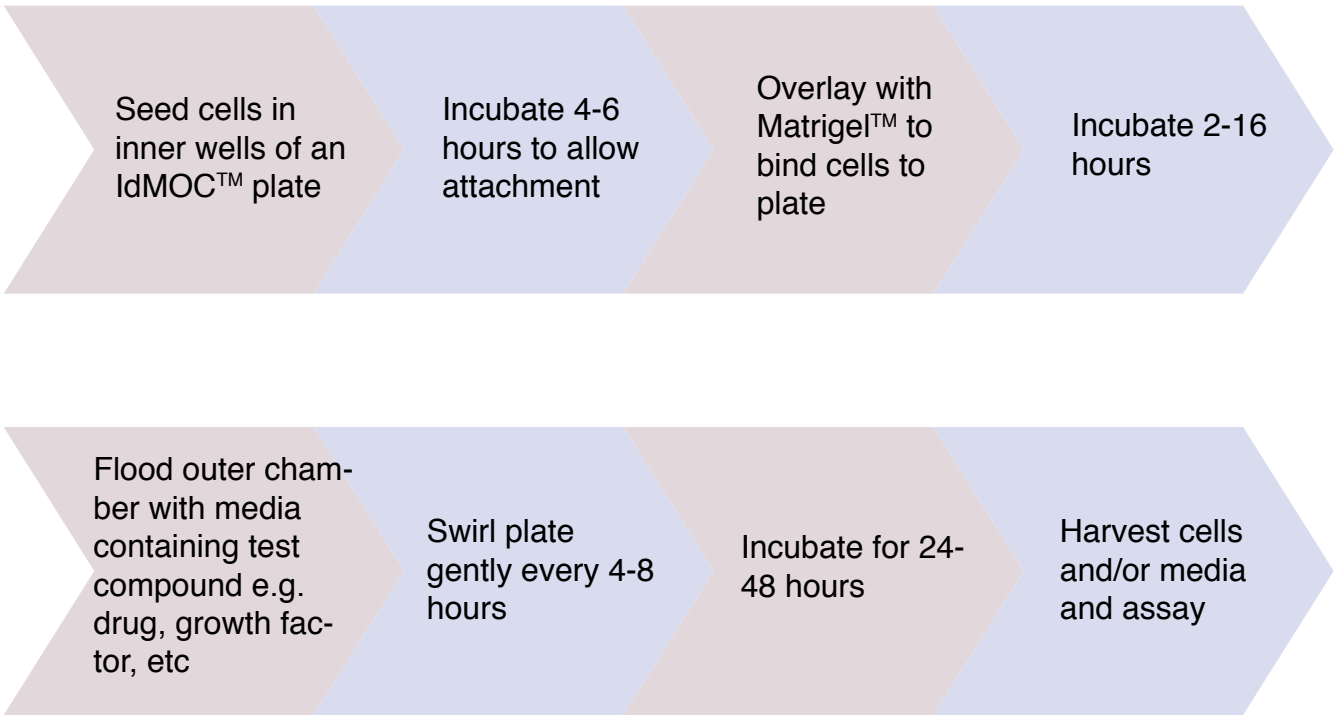
Introducing IdMOC™ Technology

Integrated Discrete Multiple Organ Co-culture

The Integrated discrete multiple organ co-culture (IdMOC™) is an in vitro experimental model for biomedical research. The IdMOC™ technology was developed based on the concept that the multiple organs in a human being (or animal) are physically separated but interconnected by the systemic circulation (i.e., blood). IdMOC™ uses a wells-in-a-well concept, with cells from individual organs seeded into each of the inner wells, and then interconnection of these physically separated cells by flooding the inner wells with an overlying medium. IdMOC™ can be used to evaluate drug toxicity, drug metabolism, drug distribution, as well as anti-cancer drugs for cytotoxicity, efficacy, and mechanism of action. It represents a more complete in vitro experimental system than the commonly used single-cell-type in vitro systems.

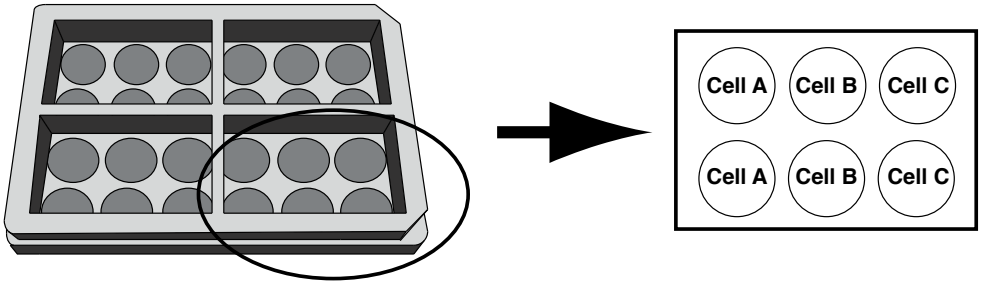
Applications:

- Toxicological Evaluation
- Drug Metabolism and Pharmacokinetics
- Pharmacology
- Cell Biology



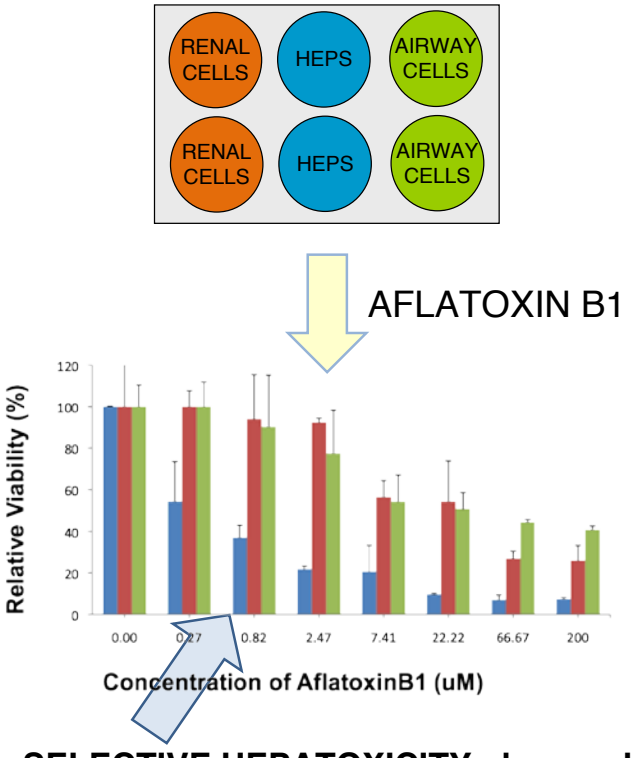
Multiple Organ Toxicity Screening

In previous in vitro systems, each cell type was studied in isolation, ignoring critical interactions between organs when metabolizing drugs. This led to the possibility of many factors such as inter-organ toxicity going unrecognized in drug evaluations. The IdMOC™ models in vivo multiple-organ interaction, thus allowing the evaluation of organ-specific effects of a drug and its metabolites. Customize your array of cell types as required. Inquire for further details.



Multi-Organ Toxicity Model using IdMOC™ plates

IdMOC Detection of Selective Toxicity



SELECTIVE HEPATOXICITY observed

Primary human cells*:

- **Kidney proximal tubule cells**
- **Liver hepatocytes**
- **Small airway epithelial cells**
- **Neuronal cells**

**Choose from our list of organ-specific cell types*

Endpoints measured:

Acute Toxicity

- Cytotoxicity (ATP content/MTT metabolism)
- Apoptosis (Caspase activity)

Sub-acute Toxicity (real-time PCR)

- Cell cycle arrest
- Oxidative stress
- DNA damage
- Inflammatory response
- Carcinogenesis

Loss of function (real-time PCR)

- Gene expression markers for organ-specific functions

Organ-specific Toxicity Screening

IdMOC™ technology represents a more complete in vitro experimental system than the commonly used single-cell-type systems. Using IdMOC™ plates, different cell types of a particular organ can be co-cultured and simultaneously treated with a test compound. This allows for cell-cell interactions and offers a drug screening model that closely mimics in vivo conditions.

Customize your array of cell types as required. Inquire for further details. We provide service for both activity-based (phenotypic) and gene expression (real-time PCR) endpoints.

Phenotypic and Gene Expression (Quantitative RT-PCR) Endpoints:

Acute Toxicity

Cytotoxicity

- ATP content
- MTT metabolism
- Enzyme release (LDH, AST, ALT)

Apoptosis

- Caspase activity
- Real-time PCR: Annexin V, CASP1, CASP3, CASP7, CASP8, CASP9, Bax, Bcl-x, FasL, TNF, TNFR1, TRAIL

Sub-acute Toxicity (real-time PCR)

Cell cycle arrest: p21Cip1, GADD153/CHOP, GADD45A, MDM2, Rb, p53

Oxidative Stress: GPX1, GSR, GSTM3, SOD1, SOD2, SOD3, DUOX1, DUOX2, NOX5

DNA Damage: ATM, RAD53, RAD23A, RAD50, XRCC1, XRCC2, ERCC1, ERCC3, UNG

Inflammatory response: MIP-1a, MIP1b, MIP2, CXCL10, GM-CSF, IL1A, 1B, IL2, IL3, IL4, IL8, IL10, IL18, TNFb, Nκ-B, iNOS, PAI-1, COX2

Hepatotoxicity Gene Expression Endpoints:

Hepatic function: ALB, GGT, SGOT, SGPT, HNF4, HMOX1

Phase I metabolism: CYP1A2, CYP2B6, CYP3A4

Phase II metabolism: NAT1, NAT2, NAT5, SULT1A1, SULT2A1, SULT4A1, UGT1A1, UGT2A1, UGT3A1, NQO1, EPHX1, CES1, TPMT

Efflux transporters: ABCB1, ABCB11, ABCC2, ABCC3, ABCC4, ABCG2

Uptake transporters: SLC10A1, SLC22A1, SLC22A7, SLCO1B1, SLCO1B3, SLCO2B1

Cholestasis: ATP8B1

Steatosis: FASN, LPL, SCD

Phospholipidosis: ASAH1, FABP1, HPN, LSS, SERPINA3

IdMOC™ Model	Human Primary Cells Include:
Hepatoxicity Model	Hepatocytes Hepatic stellate cells Hepatic sinusoidal endothelial cells Intrahepatic biliary epithelial cells
Neurotoxicity Model	Neuronal cells Astrocytes Meningeal cells Cerebellar granule cells Schwann cells Perineural cells
Cardiotoxicity Model	Cardiomyocytes Cardiac fibroblasts Aortic endothelial cells Aortic smooth muscle cells
Nephrotoxicity Model	Proximal tubular epithelial cells Cortical epithelial cells Glomerular epithelial cells Mesaglial cells Renal epithelial cells
Dermal toxicity Model	Epidermal keratinocytes Dermal fibroblasts Epidermal melanocytes-dark Dermal endothelial cells
Gastrotoxicity Model	Esophageal epithelial cells Esophageal smooth muscle cells Gastric smooth muscle cells Colonic smooth muscle cells
Pulmonary Toxicity Model*	Alveolar epithelial cells Bronchial epithelial cells Tracheal epithelial cells Small airway epithelial cells Pulmonary fibroblasts Pulmonary bronchial smooth muscle cells Pulmonary endothelial cells **Chose 6 cell types of your choice*
Ocular Toxicity Model	Ocular keratinocytes Corneal epithelial cells Retinal astrocytes Conjunctival fibroblasts Lens epithelial cells Trabecular meshwork cells
Oral toxicity Model	Oral keratinocytes Gingival fibroblasts Periodontal ligament fibroblasts
Skeletal Toxicity Model	Skeletal muscle cells Skeletal muscle satellite cells Skeletal muscle myoblasts
Breast Toxicity Model	Mammary epithelial cells Mammary endothelial cells Mammary fibroblasts
Ovarian Toxicity Model	Ovarian endothelial cells Ovarian surface epithelial cells Ovarian fibroblasts
Placental Toxicity Model	Villous trophoblasts Villous capillary endothelial cells Villous mesenchymal fibroblasts Amniotic epithelial cells Amniotic mesenchymal stromal cells
Urogenital Model	Prostatic epithelial cells Prostatic stromal cells Prostatic endothelial cells Urothelial cells Bladder endothelial cells Bladder smooth muscle cells

Anti-cancer Drug Screening

Using IdMOC™ plates, different oncogenic cell lines and their normal counterparts can be co-cultured simultaneously.

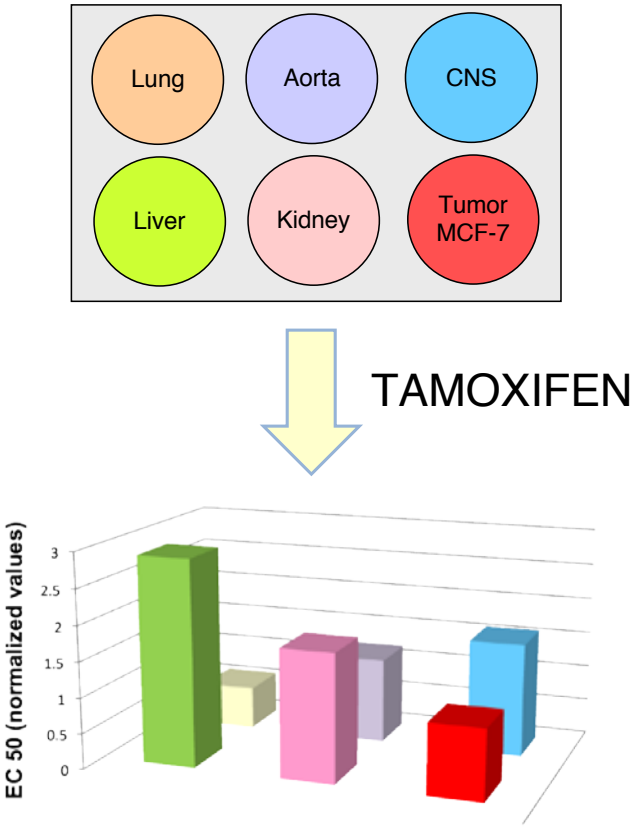
Chose from one of our IdMOC™ screening models:

IdMOC™ multi-cancer screening

IdMOC™ organ-specific cancer screening

IdMOC™ organ-specific, primary and metastatic cancer screening

IdMOC™ organ-specific, normal and oncogenic cell line screening



Li AP, Bode C, Sakai Y. Chem Biol Interact. 2004 Nov 1;150(1):129-36

Endpoints measured:

Cell viability: MTT metabolism
Cell proliferation: BrdU incorporation
Cell survival: AKT, BAX, BCL2, BCL2L1, ELK1, FOS, MYC, NFKB, TNFRSF11A
Cell cycle progression: Cyclin D1, Cyclin E1, CDK2, CDK4, P21Cip1, p27Kip1, p19Ink4d, p53, RB1
DNA repair: APC, ATM, BRCA1, BRCA2, ERCC3, MSH2, XPA, XPC
Growth factor receptors: ERB1, Her-2, ERBB3, ERBB4, FGF2R, IGF1R, IGF2R, MET
Hormone Receptors: AR, ER-α, ER-β, PPARα, PPARδ, PPARγ, RARA, RARB, RARG, RXRA, RXRB
Drug Metabolism: CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, EPHX1, GSTP1, NAT2, SOD1, SULT1E1, TPMT, ARNT
Drug Resistance: ABCB1, ABCC1, ABCC2, ABCC3, ABCC5, ABCC6, ABCG2, CLPTM1L

Simultaneous Screening for In Vitro Drug Metabolism and Toxicity

The IdMOC™ system can be used to simultaneously study drug metabolism, toxicity, distribution, stability and efficacy. Treatment of hepatocytes and non-hepatocyte cultures within an IdMOC™ plate provides a means to determine effects of drug metabolites on non-hepatocyte cultures as well as estimate drug distribution and stability. By modeling multiple-organ interactions, IdMOC™ can examine the pharmacological effects of a drug and its metabolites on target and off-target organs as well as evaluate intrinsic clearance and drug-drug interactions by measuring CYP450 induction and inhibition in hepatocytes. Listed below are the hepatic metabolism and organ-specific toxicity models using IdMOC™ plates.

Hepatic Metabolism and Organ-specific IdMOC™ Toxicity Model	Human Primary Cells Include:
Drug Metabolism and Neurotoxicity Model	Hepatocytes Neuronal cells
Drug Metabolism and Cardiotoxicity Model	Hepatocytes Cardiomyocytes
Drug Metabolism and Nephrotoxicity Model	Hepatocytes Proximal tubular cells
Drug Metabolism and Dermal toxicity Model	Hepatocytes Epidermal keratinocytes
Drug Metabolism and Gastrotoxicity Model	Hepatocytes Colonic epithelial cells
Drug Metabolism and Pulmonary Toxicity Model	Hepatocytes Small airway epithelial cells
Drug Metabolism and Ocular Toxicity Model	Hepatocytes Ocular keratinocytes
Drug Metabolism and Oral toxicity Model	Hepatocytes Oral keratinocytes
Drug Metabolism and Skeletal Toxicity Model	Hepatocytes Skeletal muscle cells
Drug Metabolism and Breast Toxicity Model	Hepatocytes Mammary epithelial cells
Drug Metabolism and Ovarian Toxicity Model	Hepatocytes Ovarian surface epithelial cells
Drug Metabolism and Placental Toxicity Model	Hepatocytes Amniotic epithelial cells
Drug Metabolism and Urogenital Model	Hepatocytes Prostate epithelial cells

Hepatocytes: *Cryopreserved human platable hepatocytes.*

Non-hepatocyte cultures: *Chose from one of several non-hepatocyte primary human cells used to establish our organ-specific toxicity models.*

Endpoints measured:

Loss of parent compound (LC-MS)

Metabolites formed (LC-MS)

Pathway identification (use of CYP inhibitors)

Cytotoxicity (MTT metabolism)

Apoptosis (Caspase activity)

Gene Expression Assays (see previous sections)

Primary Cell Cytotoxicity Assay

In vitro testing using primary cells can quickly screen for new chemical entities with serious toxicology consequences. Using primary cells derived from human tissues, the relative risk can be determined in multiple organs over a population of donors. The following in vitro cytotoxicity assays using the specified endpoints have been developed in our laboratory. With our ready inventory of human and cynomolgus monkey cells scheduling is not an issue. Contact IVAL to begin designing your study today.

Cell viability (luminescence based ATP assay)

Cell growth and survival (MTT/WST-1 metabolism)

Apoptosis (luminescence based caspase activity assay)

Oxidative Stress (luminescence based glutathione Assay)

Primary cells validated for such studies include:

- Hepatocytes (cryopreserved, human and animal hepatocytes)
- Kidney proximal tubule epithelial cells
- Astrocytes
- Aortic endothelial cells
- Small airway epithelial cells

Drug-drug Interaction Assay

The FDA recommends the evaluation of new drug candidates for drug-drug interactions. The identity of the metabolites allows the assignment of major metabolic pathways, which will facilitate experimental design for the evaluation of drug-drug interaction potential. For instance, if a drug forms phase 1 oxidation metabolites, then P450 pathways may be involved in its metabolism. Experiments concerning P450 pathway identification, P450 inhibition and induction are studies that would need to be performed. Choose your in vitro system from our inventory of liver microsomes and cryopreserved hepatocytes:

Liver Microsomes:

Human
Cynomolgus monkey
Rhesus monkey
Sprague-Dawley rat
CD-1 mouse
Beagle Dog

Cryopreserved Hepatocytes:

Human
Cynomolgus monkey

Pathway Determination Assay

Identification of the major pathways involved in the metabolism of a drug will be performed using liver microsomes in the presence of selective inhibitors for the 8 major CYP isoforms, (tabulated below). The ability of an inhibitor to inhibit metabolism of the drug would indicate that the pathway inhibited by the inhibitor is involved in metabolism. Quantification of the parent chemical and its metabolites will be performed using HPLC-LC/MS and the percent contribution of a specific CYP isoform towards metabolism of the test article is reported.

CYP enzyme	FDA recommended inhibitor	IVAL validated inhibitor
1A2	Furafylline, Alpha-napthoflavone	Furafylline
2A6	Tranylcyproamine, Methoxsalen, Pilocarpine,Tryptamine	Tranylcyproamine
2B6	Ticlopidine, Sertraline	Ticlopidine
2C8	Quercetin, Trimethoprim, gemfibrozil	Quercetin
2C9	Sulfaphenazole, Flucanozole	Sulfaphenazole
2C19	Ticlopidine	Omeprazole
2D6	Quinidine	Quinidine
2E1	Diethyldithiocarbamate	Diethyldithiocarbamate
3A4/5	Ketoconazole, Itracanozole, Troleandomycin, Verampinil	Ketoconazole

P450 Inhibition Assay

A major mechanism of drug-drug interaction is the inhibition of drug metabolizing enzymes by a drug, thereby inhibiting the metabolism of co-administered drugs, which are substrates of the inhibited pathways. This can be performed using liver microsomes (human/animal) and hepatocytes readily available at IVAL. Choose between luminescent or LC/MS validated protocols.

P450 Induction Assay

Enzyme induction is a major mechanism of pharmacokinetic drug-drug interactions. A drug that induces a specific drug metabolizing enzyme (e.g. a specific P450 isoform) would have the potential to enhance the metabolism of a co-administered drug that is a substrate of the induced pathway. Enzyme induction studies are generally performed using human hepatocytes. This approach is recommended by the U.S. FDA. Choose between validated luminescent or LC/MS assays.

Gene Expression Assay

The evaluation of the effect of a drug on gene expression profiles of the human genome, is considered a valuable technique for the definition of toxicological potential. Our laboratory has developed an extensive list of validated primer assays to allow the provision of contract research service in this area. The combined use of hepatocytes and real-time PCR techniques allows the determination of organ-specific cytotoxicity, mechanism of action, and potential to cause idiosyncratic hepatotoxicity.

High-throughput Adverse Drug Effect Assay (HTS-ADE)

In Vitro ADMET Laboratories (IVAL) now offers a proprietary high-throughput ADE assay in primary human hepatocytes. This robot assisted HTS assay provides reproducible and accurate results within 10 days of test article receipt. Our ADE assay addresses the 3 most critical questions in drug development, namely, **hepatotoxicity, CYP3A inhibition and induction**. Hepatotoxicity assay measures cell viability (ATP content) and apoptosis (caspase activity) in response to drug treatment. CYP3A4 inhibition and induction assays measure CYP3A enzyme activity in presence of a luciferin-based CYP3A4 substrate. Rifampin (inducer of CYP3A4) and ketoconazole (inhibitor of CYP3A4) serve as positive controls respectively.

ADME Assay

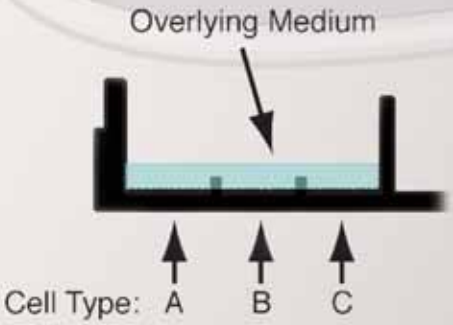
Metabolite Profiling

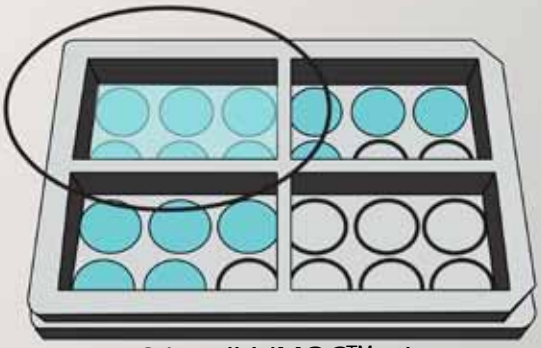
Metabolite profile comparison allows the selection of the most relevant animal species for drug properties related to human drug metabolism (e.g. safety studies). The animal species that produce a metabolite profile similar to human would be considered a relevant animal species. The use of liver microsomes allows the evaluation of metabolites formed by phase I oxidation (mainly P450 oxidation). The use of hepatocytes allows the evaluation of metabolites formed by all hepatic drug metabolizing enzyme pathways, including both phase I and phase II pathways.

Metabolic Stability

Metabolic stability is a key drug property, which is important for both drug administration regiment design as well as toxicity. Species comparison in metabolic stability allows the determination of which animal species is the most appropriate model for the estimation of human metabolic stability of the test article. The use of liver microsomes allows the evaluation of metabolic stability as results of phase I oxidation. The use of hepatocytes allows the evaluation of both phase I oxidation and phase II conjugation.

IdMOC™ Plates





24-well IdMOC™ plate

Graphic example explaining how overlying medium connects multiple cell types

The **Integrated Discrete Multiple Organ Co-culture plate (IdMOC™)** provides a method to model in vivo mulitple-organ interaction in vitro.

The IdMOC™ plate consists of multiple, inner wells within a larger interconnecting chamber. Multiple cell types are individually cultured in the inner well and the chamber is filled with a single, universal medium, allowing well-to-well communication.

The overlying medium can be analyzed for test material metabolism, and individual cell types can be evaluated for possible organ-specific bioaccumulation, cytotoxicity, and efficacy.

The patented IdMOC™ technology is now available in uncoated or collagen coated 24- and 96-well formats (2nd generation).

Product No.	Description	Unit	Price
71034	IdMOC™ (2nd gen.) Uncoated Sterile Plate, 24-well	5/pk	$500
71035	IdMOC™ (2nd gen.) Uncoated Sterile Plate, 96-well	5/pk	$500
71037	IdMOC™ (2nd gen.) Collagen Coated Sterile Plate, 24-well	5/pk	$600
71038	IdMOC™ (2nd gen.) Collagen Coated Sterile Plate, 96-well	5/pk	$600
71040	IdMOC™ (2nd gen.) Uncoated Sterile Plate, 24-well	each	$100
71041	IdMOC™ (2nd gen.) Uncoated Sterile Plate, 96-well	each	$100
71043	IdMOC™ (2nd gen.) Collagen Coated Sterile Plate, 24-well	each	$120
71044	IdMOC™ (2nd gen.) Collagen Coated Sterile Plate, 96-well	each	$120

Cell Culture Media

With over 30 years of primary cell culture experience, APSciences, associates of IVAL, has created media formulations to obtain optimal cell culturing conditions.

IVAL's latest release, LDMM, Li's Differentiation Maintenance Medium for Cryopreserved Hepatocytes, is for use in restoring gene expression and activity of hepatic functions including drug metabolizing enzymes, efflux and uptake transporters.

Improve your data confidence at any research application by using our specially formulated cryopreserved hepatocyte media for metabolism, induction, inhibition, etc;. APSciences is more than just the hepatocyte expert. Try the one-step universal recovery media, UCRM in combination with universal plating media, UPCM, to increase cryopreserved cell platability. All media are sterile-filtered, and ready to use.

Product No.	Description	Unit	Price
70005	Cryopreserved Hepatocyte Metabolism Media*, 50mL	Each	$30
70007	Cryopreserved Hepatocyte Metabolism Media*, 500 mL	Each	$160
70008	Cryopreserved Hepatocyte Inhibition Media, 50mL	Each	$30
70010	Cryopreserved Hepatocyte Inhibition Media, 500mL	Each	$160
70011	Cryopreserved Hepatocyte Induction Media, 50mL	Each	$45
70013	Cryopreserved Hepatocyte Induction Media, 500mL	Each	$230
70041	Cryopreserved Hepatocyte Metabolism Media, no salicylamide, 50mL	Each	$30
70043	Cryopreserved Hepatocyte Metabolism Media, no salicylamide, 500mL	Each	$160
70044	**UCRM**, Universal Cryopreservation Recovery Media, 50mL	Each	$60
70045	**UPCM**, Universal Primary Cell Plating Media, 50mL	Each	$45
70046	**UPCM**, Universal Primary Cell Plating Media, 500mL	Each	$135
70047	**LDMM-A**, Li's Differentiation Maintenance Media A, 50mL	Each	$120
70048	**UCMM**, Universal Culture Maintenance Media, 50mL	Each	$60
70049	**UCMM**, Universal Culture Maintenance Media, 500mL	Each	$600
70053	**LDMM-B**, Li's Differentiation Maintenance Media B, 50mL	Each	$120

**contains 3mM salicylamide*

Collagen Coated Vessels

CellAffix Cell Culture Coated Vessels take the uncertainty out of cell culturing. Available in standard and custom coating plate formats, each plate is coated with precision and tested for sterility to ensure high quality results. To maintain quality standards, CellAffix Cell Culture Coated Vessels are produced in set batch sizes and stored at 4°C prior to shipping. If your preferred plate format is not available, contact us to place a custom order.

Product No.	Description	Unit	Price
71001	CellAffix Collagen I Coated Flask, T-25 cm^2	5/pk	$60
71002	CellAffix Collagen I Coated Flask, T-75 cm^2	5/pk	$70
71003	CellAffix Collagen I Coated Flask, T-175 cm^2	5/pk	$80
71004	CellAffix Collagen I Coated Plate, 6-well	5/pk	$60
71005	CellAffix Collagen I Coated Plate, 12-well	5/pk	$60
71006	CellAffix Collagen I Coated Plate, 24-well	5/pk	$60
71007	CellAffix Collagen I Coated Plate, 48-well	5/pk	$60
71008	CellAffix Collagen I Coated Plate, 96-well	5/pk	$60
71009	CellAffix Collagen I Coated Plate, 384-well	5/pk	$130

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