Integrated discrete Multiple Organ Co-culture (IdMOC™) Plate

Advanced Pharmaceutical Sciences
Product Information

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APSciences, Inc.

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Supplied: IdMOC™ plate(s) utilizing co-culture technology of multiple wells within a larger well.

Applications:

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

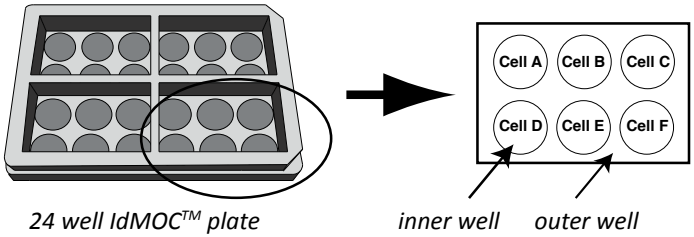
1.0 What is IdMOC™?

The Integrated discrete Multiple Organ Co-culture (IdMOC™) is an in vitro experimental model for intercellular or paracrine communication. This product was developed based on the concept that multiple organs in a human being are physically separated but interconnected by the systemic circulation.

IdMOC™ uses a wells-in-a-well concept, with cells from individual organs seeded into each of the inner wells, bound by a Matrigel™ overlay and interconnected by flooding the outer chamber with an overlying medium. IdMOC™ can be used to evaluate paracrine signaling, drug toxicity, metabolism, distribution and mechanism of action. It represents a more complete in vitro experimental system than the commonly used single-cell-type in vitro systems.

A major limitation of the use of single cell-type cultures in vitro toxicity testing is evident in the evaluation of organ-specific toxicity. Such systems ignore the critical interactions between different cell-types within an organ or between multiple organs. As a result, current models do not adequately mimic in vivo toxicological data. The Integrated Discrete Multiple Organ Co-culture (IdMOC™) technology was developed in our laboratory to overcome this major deficiency.

In the IdMOC™ plate, multiple cell types are seeded in inner wells and incubated for 24 hours to allow attachment, after which the larger, rectangular, outer well is flooded with media containing substrates or test compounds. The flooding medium permits interconnection of multiple inner wells mimicking the integration of multiple organs via the systemic circulation.



2.0 Applications of IdMOC™?

2.1 Toxicological Evaluation

- *Multi-Organ Toxicity:* The toxic potential of a drug can be evaluated in cells from multiple organs under identical experimental conditions. One example is the co-culturing of hepatocytes (liver), renal proximal tubule cells (kidney), and airway epithelial cells (lung) for the simultaneous evaluation of hepatotoxicity, nephrotoxicity, and pulmonary toxicity.

- *Organ Specific Toxicity:* Drug toxicity can be assessed in multiple cell types from a single organ (e.g. bronchial epithelial cells, alveolar epithelial cells, pulmonary capillary endothelial cells for lung toxicity) to evaluate the effects of a toxicant in the organ.

- *Metabolism dependent Toxicity:* Co-culture of metabolic competent cells (e.g. hepatocytes) and metabolically incompetent target cells (e.g. endothelial cells for vascular toxicity) to model liver metabolism followed by distribution of toxic metabolites to distal target organs for the manifestation of toxicity which occurrs in vivo

2.2 Drug Metabolism and Pharmacokinetics

- *Drug Distribution:* Differential drug distribution can be estimated in multiple cell types via drug quantification within cells. This can be performed to evaluate cell specific distribution for safety evaluation, or to demonstrate the desired targeting of a drug moiety to a specific cell type (e.g. tumor-specific targeting).

- *Multiple Organ Drug Metabolism (metabolite profiling; metabolic stability):* In vivo, drug metabolism occurs mainly in the liver but other organs may also contribute to the overall drug metabolism. Further, organ specific metabolites from one organ may be further metabolized by another organ. IdMOC thereby represents an ideal in vitro system for the evaluation of in vivo metabolic fate of a molecule where multiple organs are involved in its metabolism.

2.3 Pharmacology

- *Drug Efficacy:* The desired on-target and off-target pharmacological effects of drugs using cells from target organs as well as off-target organs can be estimated.

- *Anti-cancer Drug Screening:* Treatment of normal cells, transformed and/or metastatic cell lines with anti-cancer drugs can be evaluated for cell proliferation and other relevant markers using high throughput screening in IdMOC plates. The co-culturing of the target cancer cells with normal cells from key organs allows one to select anticancer drugs with acceptable toxic potential towards normal tissues.

2.4 Cell Biology

- *Paracrine signaling:* Evaluation of cellular factors secreted by one cell type on a different cell type within the same organ can be modeled. One example is the co-culturing of endothelial cells and smooth muscle cells to model vascular inflammation and cell proliferation.

- *Endocrine signaling:* Evaluation of the effects of a key cell type from one organ on the biology of that from a different organ. An example is the co-culturing of pancreatic islet cells and hepatocytes to model insulin-dependent glucose uptake and metabolism.

3.0 Protocol for cell seeding in IdMOC™ plates

1. In a sterile environment, remove the plate from the bag and open the lid. Seed cells in the inner wells of an IdMOC™ plate at desired cell density (see table). Add cells directly to the center of the well and do not shake.

2. Place the plates carefully in a tissue culture incubator at 37°C and 5% CO^2 and incubate for 4–6 hours to allow attachment.

3. Remove culture medium and replace with medium containing 0.25–1mg/ml BD Matrigel™ Basement Membrane Matrix (BD Biosciences, Sparks, MD). Note Matrigel™ will gel rapidly at 22°C to 35°C. Therefore thaw overnight at 4°C on ice. Mix Matrigel™ to homogeneity and dilute to desired concentration in culture medium.

4. Incubate at 37°C and 5% CO^2 for 1 hour (or overnight, as required) to allow Matrigel™ to solidify.

5. *Optional: Aspirate unbound material and rinse gently using culture medium.*

6. To initiate well-to-well communication, remove medium from individual inner wells and flood outer well with the specified volume of medium (see chart). Incorporate desired drug or chemical into the flooding medium before addition to the plate.

7. Incubate at 37°C and 5% CO^2 until required. A 24–48hr treatment period is recommended for drug evaluations.

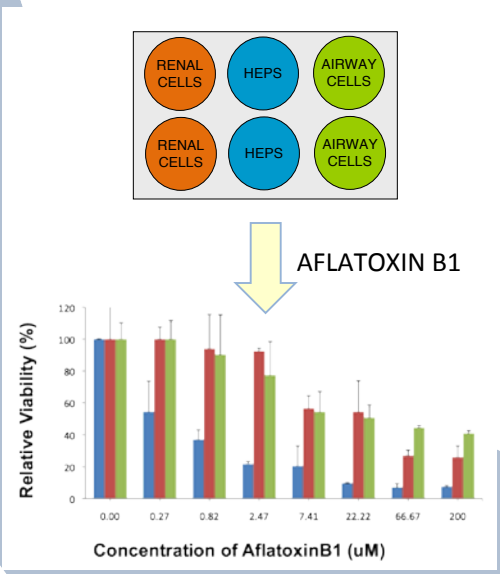
8. Swirl plate gently at regular intervals to facilitate uniform distribution of paracrine factors.

9. Harvest media and/or cells as required.

3.1 Seeding densities and flooding volumes

	FORMAT		
IdMOC™ Plate	***6-well***	***24-well***	***96-well***
Non-proliferating cells (e.g. hepatocytes)	1 x 10^6 cells/well	3 x 10^5 cells/well	5 x 10^4 cells/ well
Proliferating cells	2 x 10^5 cells/well	5 x 10^4 cells/well	5 x 10^3 cells/ well
Seeding volume (inner well)	2 ml	0.2 ml	0.06 ml
Flooding volume (outer well)	30 ml	4 ml	1.2 ml

3.2 Example of cell growth on IdMOC™ plates



Aflatoxin B1 is selectively cytotoxic to hepatocytes. Human renal proximal tubule epithelial cells, human hepatocytes and human small airway epithelial cells were cultured in IdMOC™ plates (24-well) and treated with different concentrations of Aflatoxin B1 for 48 hours. Results are shown as relative viability (mean±sd, n=3) based on cellular ATP content. Selective toxicity of Aflatoxin B1 towards hepatocytes is evident at concentrations ranging from 0.82-200µM.

4.0 References

Downloadable pdfs available at www.apsciences.com.

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