CACO-2 CELL MONOLAYER *in vitro* SCREENING TOOL FOR DRUG DISCOVERY AND MECHANISTICS: PROTOCOLS FOR CULTURE

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The Caco-2 cell monolayer model can be conveniently used for the purpose of absorption screening (Zhou *et al.*, 2012), particularly as an *in vitro* model of the intestinal barrier. This monolayer has been extensively used to determine the transport kinetics and metabolism of dietary compounds *in vitro*. As such, the Caco-2 monolayer is a useful *in vitro* screening tool for drug discovery and mechanistic studies.

An example of bioactive compounds which can be screened using this model is those having anti-diabetic effects. Type 2 diabetes mellitus (T2DM) is becoming prevalent in modern societies, and this chronic disease is estimated to reach 439 million cases by 2030 (Olokoba *et al.*, 2012). The search for compounds which have anti-diabetic effects, especially from plants, is thus an active area of research, especially in Malaysia where there is rich biodiversity. One of the mechanisms by which anti-diabetic compounds work is via inhibition of glucose transporters. Caco-2 cell monolayers have been used to investigate the effects of dietary compounds from plants on glucose transport (Manzano and Williamson, 2010; Alzaid *et al.*, 2013; Farrell *et al.*, 2013).

Therefore, the Caco-2 cell monolayer is a valuable *in vitro* model for identifying bioactive compounds related to protein transporters, such as anti-diabetic compounds which inhibit glucose transporters.

THE TECHNOLOGY

The protocols and hands-on sessions offered are optimised for the culturing of Caco-2 monolayers to screen for bioactive compounds *in vitro*, such as anti-diabetic compounds. The steps included in the protocols are summarised in *Figure 1*.

Successful glucose transport assays to identify compounds with anti-diabetic effects have been conducted using positive control glucose transport inhibitors phloretin and phloridzin by utilising the protocols outlined in this TOS.

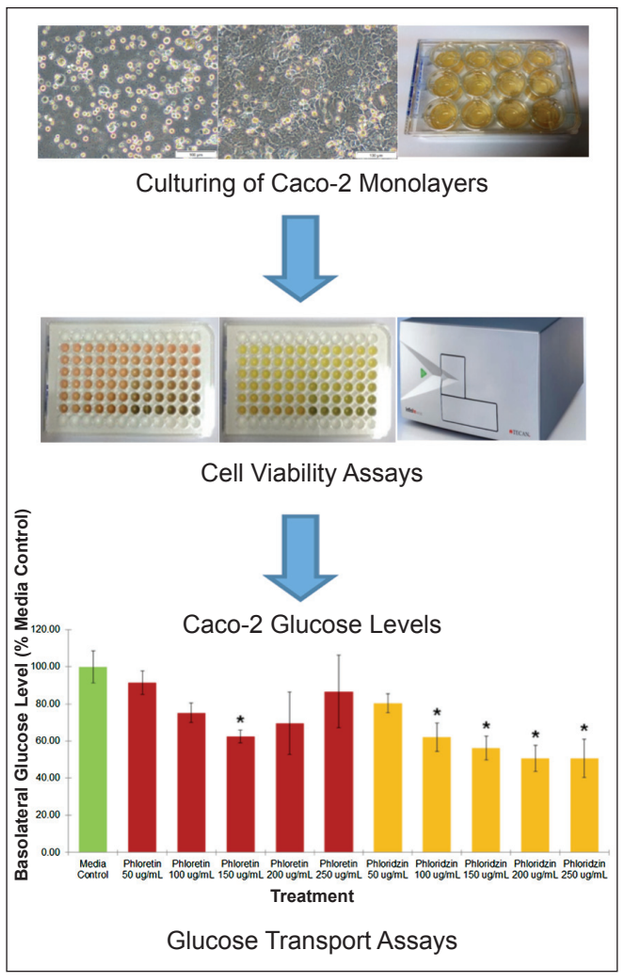

Figure 1. Workflow to screen for anti-diabetic compounds (Glucose transport inhibitors) in vitro *using Caco-2 monolayers.*

SERVICES OFFERED

The availability of a cell culture facility in the Advanced Breeding and Biotechnology Centre (ABBC), MPOB, enables the culturing of Caco-2 monolayers which requires proper aseptic techniques. Among the optimised protocols and hands-on sessions offered are:

1. Culturing of Caco-2 monolayers which involves subculturing, maintenance and

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cryopreservation of Caco-2 cells, preparation of transwell filter inserts, cell counting and seeding, maintenance of monolayers, as well as transepithelial electrical resistance (TEER) measurement to determine the integrity of the monolayers;
2. Cell viability assays to determine the maximum non-toxic dose (IC_{50}) of compounds to be tested; and/or
3. Glucose transport assays to study the effects of compounds on glucose transport.

NOVELTY, BENEFITS AND ADVANTAGES

1. Optimised protocols for culturing Caco-2 monolayers are offered.
2. Import of costly ready-to-use Caco-2 monolayers can now be avoided.
3. Caco-2 monolayers can be used as an *in vitro* screening tool for mechanism-based drug discovery, such as to identify anti-diabetic compounds.

ECONOMIC ANALYSIS

Service types	Service details	Price (RM)	
		Non-commercial	Commercial
Culturing of Caco-2 monolayers	Subculturing, maintenance and cryopreservation of Caco-cells, preparation of transwell filter inserts, cell counting and seeding, maintenance of monolayers, TEER measurement and related data analysis.	5000/session	6000/session
Cell viability assays	To include cell viability assays and related data analysis.	2000/session	2500/session
Glucose transport assays	To include glucose transport assays and related data analysis.	1000/session	1500/session

IMPACT

This TOS would enable bioactive compounds to be discovered, in addition to studying the related transport-related mechanisms. It would also promote collaborations between research institutions and universities, especially those working on potential bioactive plant extracts, herbal supplements or pure compounds.

CONCLUSION

The protocols outlined would enable drug discovery and mechanistic studies to be performed in a more cost effective manner without the need to import ready-to-use Caco-2 monolayers.

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