Utility of Automated Drug Transport Assays in 96-Well Format, using Permeable Support Systems

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Overview

Cell-based drug transport assays, such as Caco-2 and MDCK, are an essential component of ADME/Tox testing for lead compounds. The permeability and transport data they provide can determine whether or not a compound is carried forward in the drug discovery process. Current methods that use 24-well plates, run in a manual format, are no longer viable based upon the need to generate absorption data on an increasing number of compounds. By incorporating 96-well HTS Transwell® permeable supports, along with an automated robotic instrumentation, data can be generated that is more consistent when compared to manual methods, and allows research staff to perform more important, higher level functions.

Introduction

Drug transport assays play an important part in determining how a compound is absorbed into the body. Therefore, the performance of these assays is essential to help determine the ADME/Tox profile of a new drug entity (NDE). Typically, these assays have been carried out using colorectal carcinoma (Caco-2) cells, or Madin-Darby Canine Kidney (MDCK) cells in 24-well plates. However, due to the fact that ADME/Tox testing is now moving further upstream in the drug discovery process, a greater number of lead compounds are now being tested in an effort to fail NDEs with negative profiles earlier and in a more cost-effective manner. To meet the demands for higher throughput and reduced processing time, we present an automated drug transport assay using either Caco-2 or MDCKII/MDR1 cells in 96-well Permeable Supports. The entire assay process was automated, including cell dispensing, media exchanges, and compound addition and removal, using simple, yet robust robotic instrumentation. A two-part permeable support system, incorporating an insert plate, and receiver plate, was used in order for manipulations to be performed without the need to separate the parts of the system. The matrix used to validate the automated process were Transwell® Electrical Resistance (TER), and Lucifer Yellow and Rhodamine 123 permeability. All automated methods were done in parallel to manual methods for comparison. Results show that the automated processes could deliver results that are equal to, or more consistent than, manual processing, while reducing the overall experimental time. Thus, by automating the drug transport assay, one increases efficiency without the loss of data quality or integrity.

BioTek Instrumentation

The EL406™ Combination Washer Dispenser offers fast, accurate media removal and plate washing capabilities through its Dual-Action™ Manifold. It also offers reagent dispensing capabilities through the use of its peristaltic or syringe pumps, with volumes ranging from 1 to 3000 µl/well. The instrument was used for cell dispensing, media exchange and removal, as well as dispensing of buffer and reagents to the Transwell permeable supports. The small footprint of the instrument allows for easy insertion into existing laminar flow hoods to ensure sterile manipulations.

BioTek Detection

The Synergy™ H4 Hybrid Multi-Mode Microplate Reader combines a filter-based and monochromator-based detection system in the same unit. The filter-based system was used to read the fluorescent Lucifer Yellow signal using a 485/20 nm excitation filter, 530/25 nm emission filter, and 510 nm cutoff dichroic mirror and Rhodamine 123 signal using a 530/25 nm excitation filter, 590/35 nm emission filter, and 550 nm cutoff dichroic mirror.

Transwell Permeable Support System

Corning HTS Transwell-96 permeable supports are designed for high-throughput applications to examine cell polarization, drug transport, toxicity, and chemotaxis in vitro. The HTS format allows for all 96 inserts to be handled as a single unit making it an ideal tool for automated high throughput studies.

Drug Absorption Assay Protocols

Drug Transport Analysis

1. The EL406 is able to dispense MDCK and Caco-2 cells, and perform media transfers easily and efficiently without disturbing cell monolayers, as evidenced by TEER and Lucifer Yellow results.
2. Papp values, with less variability among replicates, lead to more appropriate P-glycoprotein efflux ratios and conclusions.
3. Corning HTS Transwell-96 permeable supports provide an easy to use, and flexible method for studying drug transport.
4. The combination of BioTek’s instrumentation, and Corning’s Transwell plates, create an ideal solution for performing high-density, automated drug transport assays.

Conclusions

Figure 1 – MDCK Cell Preparation and Assay Protocol.

Figure 2 – Caco-2 Cell Preparation and Assay Protocol.

Figure 3 – Average TEER values across three runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

Figure 4 – Average apparent permeability values (Papp) for Lucifer Yellow across three runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

Figure 5 – Average A-B and B-A Papp values for Rhodamine 123 across three runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

Figure 6 – Average calculated efflux values for Rhodamine 123 across three runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 or manual method (n=40).

Figure 7 – Average TEER values across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40).

Figure 8 – Average apparent permeability values (Papp) for Lucifer Yellow across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40).

Figure 9 – Average A-B and B-A Papp values, and efflux values for uninhibited and inhibited Rhodamine 123 across two runs of 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40). Uninhibited apical wells for B-A transfer contained HBSS buffer with 1% DMSO, while inhibited apical wells contained 10 µM Cyclosporin A in HBSS buffer.

Figure 10 – Average apparent permeability values (Papp) for Lucifer Yellow across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40).

Figure 11 – Average apparent permeability values (Papp) for Lucifer Yellow across two runs for 0.4 and 1.0 µM Transwell permeable supports processed using EL406 (n=40).

Lower Rhodamine 123 apical-basolateral (A-B) values and higher basolateral-apical (B-A) values for EL406 processed plates demonstrate a more intact cell layer and higher functioning P-glycoprotein, respectively, when compared to manually processed plates.