

Technical Bulletin #415

Use of Automation-Compatible 24-Well Insert System for Various Cell-Based Assays

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Introduction

In recent years, the use of cell-based assays for drug screening applications has increased tremendously. However, some cell-based assays are difficult to use in a highly automated screening laboratory setting due to special handling conditions required for cells, or general format incompatibility of the assay platform with automation equipment. This is particularly true of membrane-based cell assays. Current microporous membrane systems for cell-based assays utilize individual inserts which are not easy to use with automation equipment. To overcome these barriers, we have developed an automation compatible 24-well insert system for use in cell-based assays and tested the system in several cell-based applications.

Using a unique 24-well insert format, we have produced insert systems incorporating either 1, 3, or 8 μm PET (Polyethylene terephthalate) membranes for different cell-based applications. The 1 μm system has been tested for its ability to support the differentiation of Caco-2 cells used for drug permeability studies. The 3 μm system has been tested for its ability to support neutrophil migration in response to a chemotactic gradient. While the 8 μm system has been tested for its ability to support an assay for tumor cell invasion through an extracellular matrix protein layer. The data presented here demonstrates the utility and performance of this system in several commonly used cell-based assays.

Materials and Methods

Caco-2 Assay (1 μm PET membrane)

A BD Falcon™ HTS Multiwell™ Insert System with 1 μm PET membrane was prepared with fibrillar collagen. Caco-2 cells were seeded onto the fibrillar collagen inserts at a density of 200,000 cells/insert in Basal Seeding Media supplemented with Mito+ Serum Extender (BD Biosciences). Media volumes of 0.5 ml above and 1 ml below the insert were used. Cells were then cultured for 24 hours. After 24 hours, media was removed and changed to Entero-STIM media supplemented with Mito+ Serum Extender. Cells were then cultured for another 48 hours. At the end of this time, media was removed and Caco-2 cell barrier function was assessed by Mannitol Permeability measurement. Briefly, ^3H -Mannitol was added to PBS

buffer above the cell layer. Cells were then incubated for 30-60 minutes at room temperature and the amount of ^3H -Mannitol passing through the cell layer was determined by liquid scintillation counting. Using this data, the Permeability coefficient (P_c) for Mannitol was calculated. The data is presented in Figures 1 and 2.

Neutrophil Chemotaxis Assay (3 μm PET membrane)

Human neutrophils were obtained from whole blood from a healthy donor by progressive dextran sedimentation and Ficoll gradient procedures. Freshly isolated human neutrophils were added to a BD Falcon HTS Multiwell Insert System with a 3 μm PET

membrane placed in a 24-well plate. Neutrophils were added at a density of 1×10^6 cells/insert. Buffer containing 1×10^{-8} M fMLP (f-Met-Leu-Phe) was used as chemoattractant in the lower chamber. Neutrophils were allowed to migrate for 90 minutes. At that time, cells were fixed, stained, and counted by microscope to determine % cell migration. The data for these experiments is presented in Figure 3.

Tumor Cell Invasion Assay (8 μm PET membrane)

A 24-well Insert System using an 8 μm PET membrane was obtained and coated with BD Matrigel™ Basement Membrane Matrix. Inserts were rehydrated with DMEM (Dulbecco's Modified Eagle's Media) for 2 hours at room temperature prior to use. After rehydration, media was removed and either 3T3 or HT 1080 cells (at 100,000 cells/insert) were added to the top of each insert chamber in DMEM + 0.1% BSA. Using 3T3 conditioned media as chemoattractant, cells were incubated at 37°C for 24 hours. After incubation, non-invading cells were carefully removed from the top of each insert with a cotton swab. Cells were fixed, stained, and photographed microscopically to determine the extent of cell migration. Results are presented in Figure 4.

Results and Discussion

By using the BD Falcon™ HTS Multiwell Insert System with a 1 µm PET membrane in conjunction with other biological components, Caco-2 cells can be induced to form a cell barrier suitable for automated compound permeability measurements in 3 days (Figure 2). The P_c for Mannitol was $4-6 \times 10^{-6}$ cm/sec in this system. Use of a 3 µm PET membrane in the Insert System allows for the development of an automated assay to look at neutrophil migration. The data in Figure 3 shows how neutrophils can be induced to migrate in response to a chemoattractant and measured in the Multiwell Insert System. The use of an 8 µm PET membrane, in conjunction with a biological protein matrix, allows for the development of an automated tumor cell migration assay. In this assay, invasive tumor cells (HT 1080) can be induced to migrate in response to a chemoattractant, while non-invasive cells (3T3) will not migrate in response to a chemoattractant in this system (Figure 4).

By developing an automation-compatible 24-well Insert System and testing it in several cell-based assay systems, we believe that we have provided a system whereby many commonly used insert-based cell assays may be adapted to an automated laboratory setting. We have demonstrated the utility of this system in several cell-based assays (Caco-2 Compound Permeability, Neutrophil, and Tumor Cell Migration). By taking advantage of this 24-well insert platform, it will be possible to increase the efficiency, productivity, and throughput of many membrane-based assays.

BD Falcon HTS Multiwell Insert System

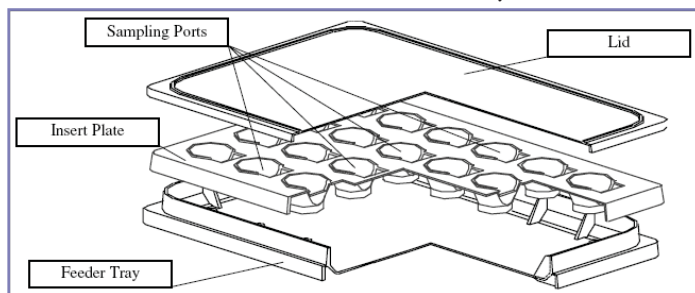


Figure 1: The BD Falcon HTS Multiwell Insert System features generous access ports and extended insert plate flanges for easy handling by robotic equipment, with or without the lid in place, a Feeder Tray to assist in uniform cell culturing, and a non-directional lid for easy lid placement.

Caco-2 Mannitol Permeability Assay

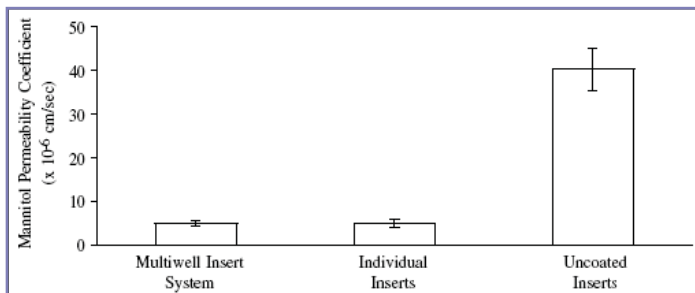


Figure 2: A 3-day Caco-2 assay was performed on both individual inserts (1 µm PET membrane) and 24-well HTS Insert System (1 µm PET membrane) coated with fibrillar collagen as described in Methods. A negative control of Caco-2 cells grown on an uncoated insert was also done. Data shown is mean of n=6 (Insert System or individual inserts) or n=3 (uncoated inserts) ± S.D.

Neutrophil Chemotaxis Assay

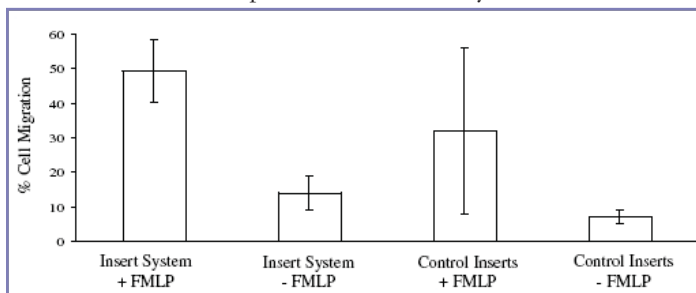


Figure 3: Freshly isolated neutrophils were tested for their ability to migrate through either individual inserts or Insert Systems (3 µm PET membrane) in response to a chemoattractant. Data shown is mean of n=6 (Insert System) and n=3 (individual inserts) ± S.D.

Tumor Cell Invasion Assay

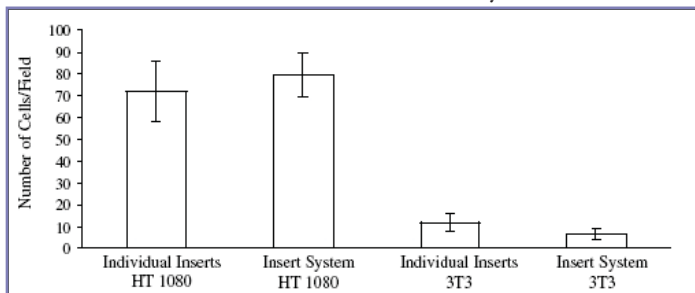


Figure 4: Both individual inserts and Insert System (8 µm PET membrane) were coated with BD Matrigel™ Matrix. Cells were added to the inserts and allowed to migrate in response to a chemoattractant for 24 hours. Data shown is mean of n=4 (Insert System) or n=2 (individual inserts) ± S.D.

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