# Technical Bulletin #419

# Design and Evaluation of an Automation-Compatible Multiwell Insert for Cell-Based Assays

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## Introduction

In recent years, the use of cell-based assays for drug discovery screening has increased tremendously, but some cell-based assays remain difficult to automate due either to special cell handling conditions or to general format incompatibility with robots. This is particularly true of membrane-based cell assays where separate housings (or inserts) support individual membranes. These inserts are difficult to manipulate, making automation complicated and expensive. To resolve this problem, Corning Life Sciences has developed an automation-compatible 24-well insert system and we have tested the system in several cell-based applications. The following data shows many of the design features and performance characteristics.

## Materials and Methods

**Design**

To design an automation-compatible Multiwell Insert System suitable for use in membrane-based cell assays, it was necessary to optimize for many of the following characteristics:

- Ease of set-up or use
- Ease of robotic manipulation
- Fluid handler access to the upper and lower chambers for sampling
- Prevent cross-contamination between chambers
- Consistent performance in membrane-based assays

This was done using an iterative procedure and the Autodesk® Mechanical Desktop installed on a desktop PC. Manufacturing equipment was then constructed and the insert system components were made.

**Evaluation**

The system was designed and tested for several physical performance characteristics (**Figures 1-4**) as well as several cell-based assays (**Figures 5**):

***Caco-2 Assay (1.0 µm PET membrane)*** - A 24-well Multiwell Insert System with a 1.0 µm PET membrane was coated with fibrillar collagen. Caco-2 cells were seeded onto these coated inserts using Basal Seeding Medium supplemented with Mito+ Serum Extender (Corning Life Sciences). Cells were cultured for 24 hours and then the medium was removed and changed to Intestinal Epithelium Differentiation Medium supplemented with Mito+ Serum Extender. Cells were cultured for another 48 hours. The medium was then removed and Caco-2 barrier function was assessed by Mannitol permeability measurement.

***Neutrophil Chemotaxis Assay (3.0 µm PET membrane)*** - Human neutrophils, obtained from healthy donor whole blood using progressive dextran sedimentation and Ficoll gradient procedures, were added to a 24-well Multiwell Insert Plate with a 3.0 µm PET membrane in a 24-well plate (Corning Life Sciences). Buffer containing 1 x $10^{-8}$ M fMLP (f-Met-Leu-Phe) was used as a 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.

***Tumor Cell Invasion Assay (8.0 µm PET membrane)*** - A 24-well Multiwell Insert System with an 8.0 µm PET membrane was coated with Corning® Matrigel® Basement Membrane Matrix (Corning Life Sciences) and then 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 were added to the top of each insert chamber. Using 3T3 conditioned media as a chemoattractant, cells were incubated for 24-hours. After incubation, non-invading cells were carefully removed from each insert with a cotton swab. Migrated cells were fixed, stained, and photographed microscopically to determine the extent of migration.

## Results and Discussion

As shown in **Figures 1 and 2**, the Falcon® Multiwell Insert System is composed of a PET (polyethylene terephthalate) Multiwell Insert Plate, Feeder Tray, and non-directional Lid. This material was chosen for its excellent cell compatibility and optical characteristics. The Insert Plate is designed for use with the standard Falcon 24-well plate.

As can be seen in **Figure 3**, this system has many features designed to facilitate automation. Twenty-four inserts were integrated into a one-piece design to facilitate manipulation. Flanges on each component have at least 6 mm vertical gripping surface. The Insert Plate flange can be gripped with the lid on and is a readable surface for labeling or identification. Further, the inner edge of this flange is beveled to aid alignment during assembly. The Lid is rectangular, free of interior features, smooth on top for vacuum delidders, and has minimal system overhang. The assembled system height is 2 mm higher than a Falcon 24-well plate and lid.

**Figure 3** also shows vertical distances and X-Y locations of the sampling port and well centerlines, referenced from the A1 corner. The D-shaped flat-sided Insert wall provides a 4.0 mm sampling port width. This generous port permits fluid handler access to the lower chamber using up to 1000 µl pipet tips without removing the Insert Plate.

**Figure 4** provides some performance characteristics of the Falcon Multiwell Insert System. Contamination from capillary action between upper and lower chambers is minimized by the addition of concentric rings to the lower outside of each insert wall. Feeder Tray ribs, originally designed as side wall supports, were observed to reduce liquid sloshing during system movement. Assay liquid volumes, procedural steps, and durations were used as a baseline to determine final component sizes, membrane seal requirements, etc. Liquid working volumes were then maximized to eliminate process steps such as cell feeding, which resulted in more robust assays.

**Figure 5** demonstrates the use of the Falcon Multiwell Insert System in several cell-based assays. These systems performed as expected in both cell barrier and cell migration assays and were equivalent to appropriate individual insert controls. Specifically, Falcon Multiwell Insert Systems supported Caco-2 cell barrier formation and migration of either neutrophils or tumor cells (HT 1080).

## Conclusion

We have demonstrated the utility and performance characteristics of the Falcon Multiwell Insert System in several cellbased assays. We believe this system provides a means to automate many commonly used membrane-based cell assays and increase the efficiency, productivity and throughput of these assays in the drug discovery process.

Falcon Multiwell Insert System

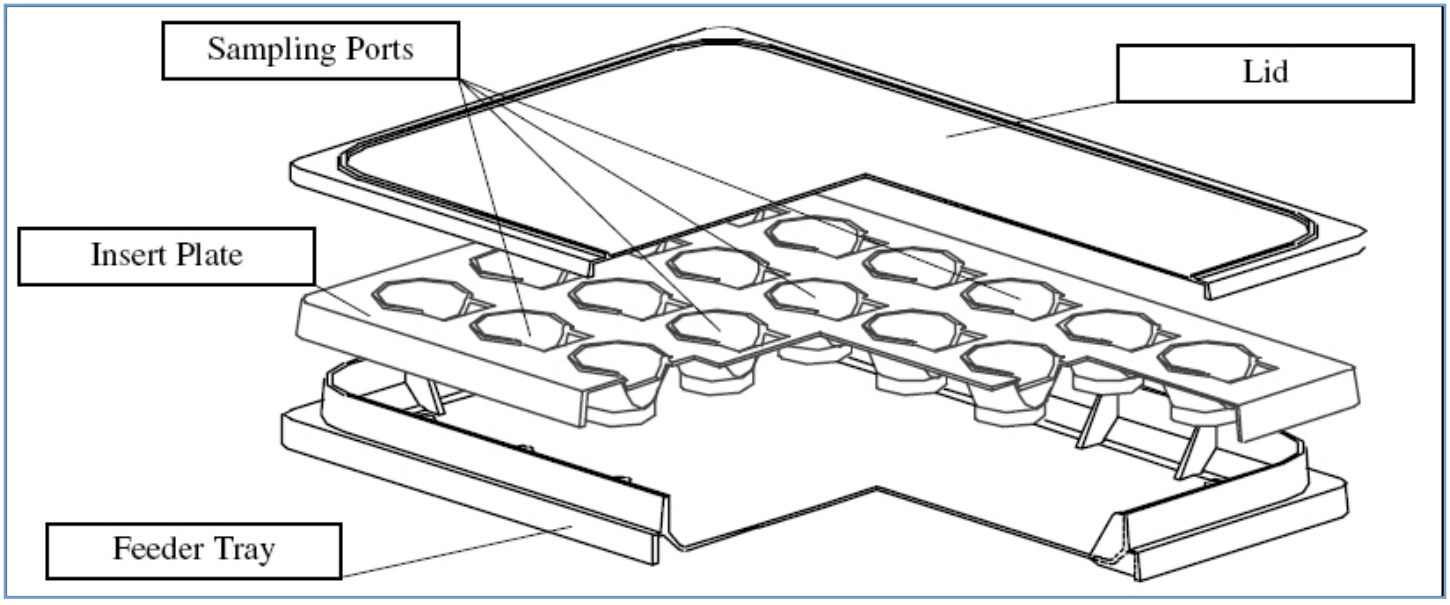



*Figure 1: The Falcon Multiwell Insert System features generous access ports and extended insert plate flanges for easy handling by robotic equipment, with or without the lid in place, a Feeder Tray to assist in uniform cell culturing, and a non-directional lid for easy lid placement.*

## Falcon® Multiwell Insert System Basic Dimensions

**LID:**

Material: PET (Polyethylene Terepthalate)

Length: A = 129.57 mm (5.101 inches)

Width: B = 86.82 mm (3.418 inches)

Height: C = 8.20 mm (0.323 inches)

**INSERT PLATE HOUSING:**

Material: PET (Polyethylene Terepthalate)

Length: D = 127.61 mm (5.024 inches)

Width: E = 85.01 mm (3.347 inches)

Height: F = 18.14 mm (0.714 inches)

***Insert Well:***

Top Interior Diameter: 12.50 mm (0.492 inches)

Bot. Int. (Membrane) Dia: 6.50 mm (0.256 inches)

Bot. Ext. Diameter: 10.00 mm (0.394 inches)

Total Well Depth: 18.14 mm (0.714 inches)

Well to Well Distance: 19.30 mm (0.760 inches)

Sampling Port Length: 9.50 mm (0.37 inches)

Sampling Port Width: 4.00 mm (0.16 inches)

**FEEDER TRAY:**

Material: PS (Polystyrene)

Length: G = 127.86 mm (5.034 inches)

Width: H = 85.47 mm (3.365 inches)

Height: I = 19.94 mm (0.785 inches)

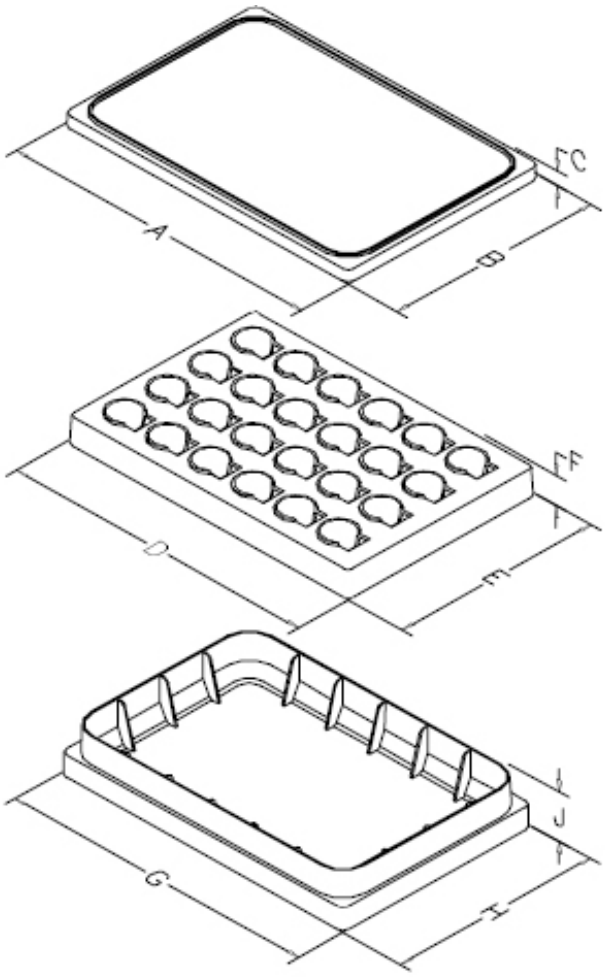



*Figure 2: Composed of three components, the Falcon Multiwell Insert System has been designed to be compatible with common laboratory automation. Basic dimensions are shown above.*

## Falcon Multiwell Insert System Programming Dimensions

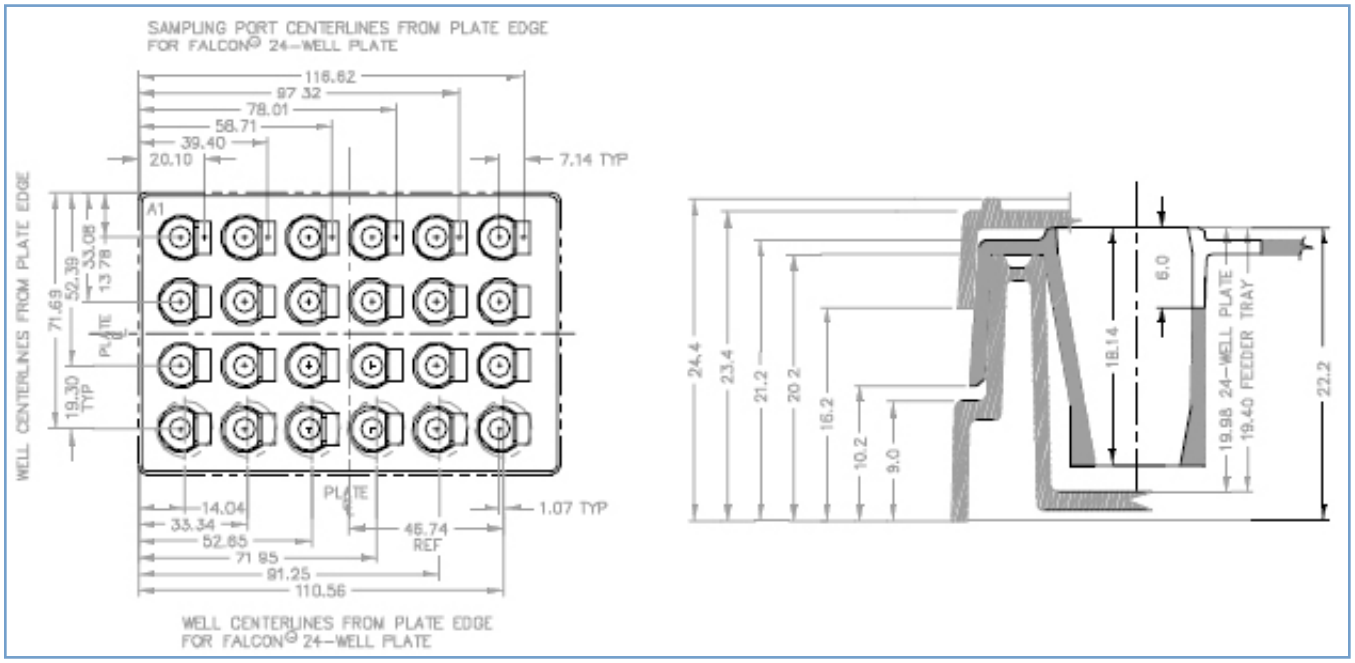



*Figure 3: The Falcon Multiwell Insert System has been designed to allow for optimal access to both upper and lower chambers. A side skirt suitable for automation is included in the design. The Falcon Multiwell Insert System is also designed such that lot components can be manipulated either as a unit or by any combination of sub-components.*

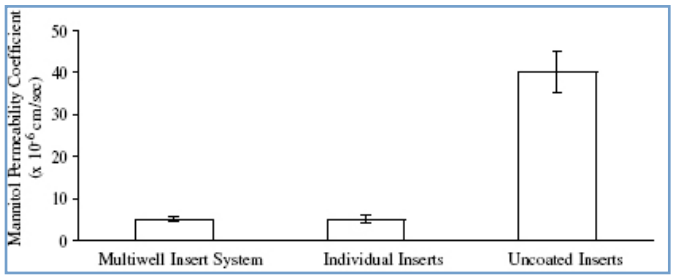
## Falcon® Multiwell Insert System Performance Characteristics

| Performance Characteristics | Tested Parameters | |
|---|---|---|
| Membrane seal durable for typical assay time periods | Membrane seal integrity maintained in media at incubation temperatures | Seal maintained for more than 3 weeks |
| Insert chamber holds appropriate media volume for assays | Cross contamination of upper/lower chamber | Insert chamber holds up to 500 µl without spill-over |
| No wicking of liquid from bottom chamber into insert chamber | Cross contamination of upper/lower chamber | Feeder tray holds up to 35 ml and 24-well plate holds up to 1.4 ml in each well without wicking |
| System holds liquid for cell growth cell assays without spillage upon agitation | Cross contamination of upper/lower chamber with agitation | Agitation up to 100 rpm possible without cross contamination with 0.5/1.4 mls in upper/lower chambers |
| System compatible with Endohm meter for measuring electrical resistance | Fit with WPI Endohm Multiwell Ohmmeter | Compatible with Endohm meter |
| Good Stacking | Deviation from 90° | <2° lean with a stack of 10 systems |
| System maintains sterile environment suitable for conducting assays | Presence of bacteria, fungi, mycoplasma | Tested and found negative |
| Access to upper/lower chambers | Access by 200 & 1000 µl pipet tips, and 1 ml, 5 ml, 10 ml, 25 ml pipets | Able to dispense and aspirate top & bottom chamber with 200 & 1000 µl pipet tips, and 1 & 5 ml pipets. Able to dispense with 10 and 25 ml pipets |

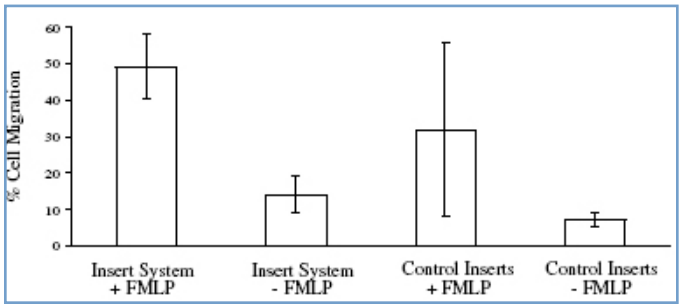
*Figure 4: Several performance characteristics of the system were determined, using various methods. All tests involving liquid were prepared with appropriate assay or cell culture media.*

## Performance of the Falcon Multiwell Insert System in Cell-Based Assays

Caco-2 Mannitol Permeability Assay




Neutrophil Chemotaxis Assay




Tumor Cell Invasion Assay

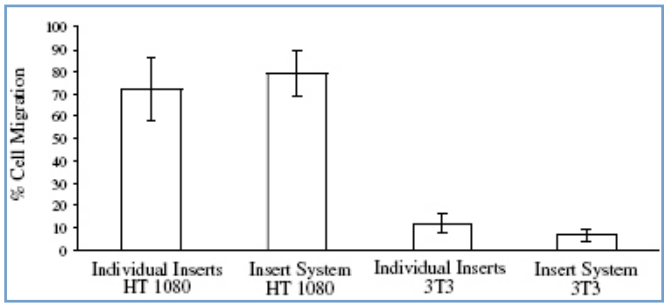



*Figure 5: The Falcon Multiwell Insert Systems (1.0, 3.0, and 8.0 µm PET membranes) were used in several cell-based assays as described above. Falcon Multiwell Insert Systems performed as expected and compared favorably to assays performed in comparable individual Falcon inserts.*

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