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Application Note

Evaluation of MultiScreen®-MIC Plates in Chemotaxis Assays

Chemotaxis assays for better lead identification in cancer drug discovery

In an effort to rapidly identify leads in the pursuit of new drugs, cell-based assays have attained tremendous importance in pre-screening of compounds. These screening programs are increasingly focused on incorporating functional cell-based assays in primary and secondary screening stages of these compounds. These assays have the potential to provide valuable information early in the drug discovery process and result in better lead identification from a functional perspective. Many drugs under development in cancer drug discovery are directed at altering the metastatic properties of cancer cells.

The premise of the cell-based assays described in this paper is that cells migrate in response to chemotaxis, the movement of cells in response to a concentration gradient set up here by a chemoattractant. Development of high throughput screening (HTS) cell-based assays that are designed to measure effects of lead compounds on processes such as metastasis can pose a challenge.

The MultiScreen®-MIC (Migration, Invasion, and Chemotaxis) plate is a 96-well sterile and disposable device designed to support assays that can model metastasis using chemotaxis. These plates are available in three pore sizes (3, 5 and 8 µm) for use with a variety of suspension and adherent cell lines.

Chemotaxis assays performed with MultiScreen®-MIC plates

The reproducibility of chemotaxis assays was assessed on various membrane pore sizes of MultiScreen®-MIC plates.

Chemotaxis of suspension cell lines HB-124 (mouse hybridoma cell line) and K-562 (human chronic myelogenous leukemia cell line) on a 3 µm membrane pore size MultiScreen®-MIC plate was evaluated. The chemotaxis profiles of highly migratory adherent cell lines MDA-MB-231 (human breast cancer cell line) and HT-1080 (human fibrosarcoma cell line) were evaluated on 5 µm membrane pore size MultiScreen®-MIC plates. Finally, the effect of two inhibitors (Cytochalasin D and Tamoxifen) on MDAMB-231 (human breast cancer cell line) chemotaxis was also evaluated.

Experiments were conducted on different lots and on different experimental days to assess the effects of these variables on assay outcomes. Consistent inter-plate, intra-plate, inter-lot, and intra-lot data was obtained with MultiScreen®-MIC plates using the recommended protocol conditions for these assays. Percent chemotaxis was consistent and reproducible in response to 10 percent serum for HB-124 (15%) and K-562 (2%), with remarkable chemotaxis in MDA-MB-231 cells. HT-1080 results varied by assay.

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Materials and Methods

Materials

- Cell Lines:
  - HB-124 (mouse hybridoma cell line)
  - K-562 (human chronic myelogenous leukemia cell line)
  - MDA-MB-231 (human breast cancer cell line)
  - HT-1080 (human fibrosarcoma cell line)
- Cell Culture Media:
  - Dulbecco's moidified Eagle's medium (DMEM), high glucose (Sigma-Aldrich® cat D5796)
  - RPMI-1640 medium with L-glutamine and sodium (Sigma-Aldrich® cat R8758)
  - MEM non-essential amino acid solution 100x (NEAA) (Sigma-Aldrich® cat M7145)
  - HEPES buffer 1 M pH 7.0 – 7.6 (Sigma-Aldrich® cat H0887)
  - L-glutamine-penicillin/streptomycin solution (Sigma-Aldrich® cat G1146)
  - Sodium pyruvate solution 100mM (Sigma-Aldrich® cat S8636)
  - Fetal bovine serum (FBS)
- Instruments:
  - Fluorescent Plate Reader
  - MultiScreen®-MIC plates (figure 1)

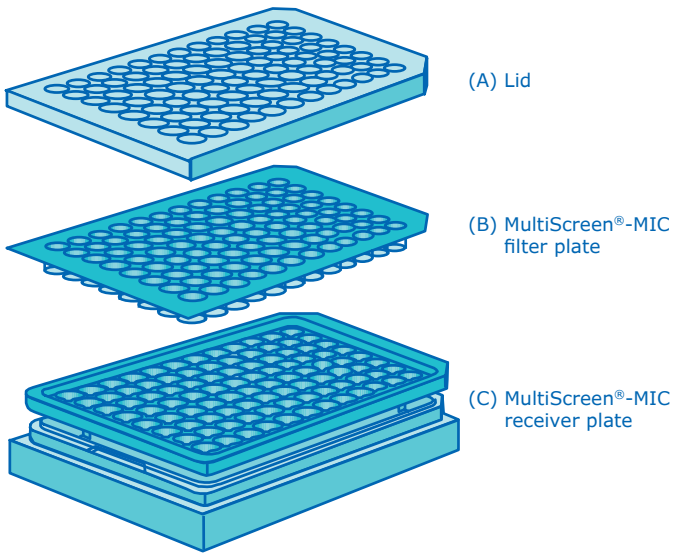

Figure 1. MultiScreen®-MIC plate components.

Methods

Cell culture:

- HB-124, K-562, and HT-1080 cells were cultured and propagated in DMEM, high glucose, supplemented with 10% FBS, 1x HEPES, 1x NEAA, 1x L-glutamine-penicillin/streptomycin.
- MDA-MB-231 cells were cultured and propagated in RPMI, supplemented with 10% FBS, 1x HEPES, 1x NEAA, 1x L-glutamine-penicillin/streptomycin.

  Cells were passaged once a week at 100% confluency and incubated at 37 ºC, 5% CO2.

Assay set-up:

1. Chemotaxis assay with suspension cells

Cell expansion and preparation

a. HB-124 and K-562 cells were expanded into T-75 flasks for 3-5 days before the experiment to reach 80-90% confluence one day prior to assay.

b. Cells were centrifuged at 120 x g for 8 minutes at room temperature to pellet, resuspended in serum free medium containing 0.2% BSA (v/v), and starved overnight at 37 ºC, 5% CO2.

c. Cell counts were adjusted to 106 cells/mL in serum free medium containing 0.2% BSA, ensuring >90% cell viability.

Chemotaxis assay on MultiScreen®-MIC plates

a. Filter plates and membranes were separated and placed into a sterile single well tray.

b. Cells were added to upper wells in the MultiScreen®-MIC filter plate, ensuring even distribution across the membrane, and chemoattractants were added to lower wells in the MultiScreen®-MIC receiver.

c. MultiScreen®-MIC filter plates and receiver plates were gently assembled, to avoid disturbing the concentration gradient.

d. The MultiScreen®-MIC plate assembly was incubated 4 h at 37 ºC. Plates were not shaken during incubation.

Fluorescence labeling

a. During incubation, 1x PBS, pH 7.4 prewarmed to 37 ºC was equilibrated to room temperature and used to prepare a 4 µM solution of calcein AM.

b. Chemotaxis assay plates were incubated for 4 h and removed from the incubator without shaking or tilting. MultiScreen®-MIC filter plates were gently removed and discarded.

c. After adding reagents to the MultiScreen®-MIC receiver, plates were incubated for 1 h at 37 ºC.

2. Chemotaxis assay with adherent cells

Cell expansion and preparation of adherent cells for chemotaxis assay

a. MDA-MB-231 and HT-1080 cells were expanded into T-75 flasks for 3-5 days before the experiment to reach 80-90% confluence one day prior to assay and starved overnight at 37 ºC, 5% CO2 in serum free medium containing 0.2% BSA (v/v).

b. Cell counts were adjusted to 106 cells/mL in serum free medium containing 0.2% BSA, ensuring >90% cell viability.

Setting up chemotaxis assay on MultiScreen®-MIC plates with adherent cells

a. MultiScreen®-MIC filter plates were separated out and placed into a sterile single well tray to protect the membrane prior to setting up the experiment.

b. Cells were added to upper wells in the MultiScreen®-MIC filter plate, ensuring even distribution across the membrane, and chemoattractants were added to lower wells in the MultiScreen®-MIC receiver.

c. (c) Inhibitors (20 µm Cytochalasin D and 50 µm Tamoxifen) were added to cells prior to loading in upper wells.

d. (d) Top and bottom plates were gently assembled to avoid disturbing the concentration gradient.

e. (e) The MultiScreen®-MIC plate assembly was incubated for 4 h at 37 ºC.

Staining procedure for adherent cell chemotaxis assay and chemotaxis inhibition assay plates

a. Towards the end of the incubation period, staining solutions were set up from the Hema-3 stain kit in MultiScreen®-MIC receiver plates, dispensing 150 µl of fixatives, Solution I and Solution II, and MilliQ® water in separate plates.

b. After incubation, chemotaxis plates were removed from the incubator without shaking or tilting.

c. Unmigrated cells in upper wells were removed with a cotton swab using gentle pressure, taking care not to puncture the membrane.

d. After swabbing all wells, upper wells were gently rinsed with 1x PBS.

e. MultiScreen®-MIC filter plates were stained and passed through the staining solutions.

Image analysis of stained invasion assay or invasion inhibition assay plates.

a. The experimental plates were counted using a Zeiss Axioplan-2 microscope equipped with KS300 3.0 automated cell counting software. A sample of the membrane surface area (11%) was counted and the cell number was extrapolated to the entire membrane (0.3 cm2 surface area).

Results

1. Chemotaxis assay with suspension cells.

The uniformity of chemotaxis values obtained was assessed in experiments performed on the same day using different lots. Figure 2 demonstrates values obtained in experiments performed with HB-124 and K-562 cells in response to 10% serum or 0.2% BSA as a chemoattractant.

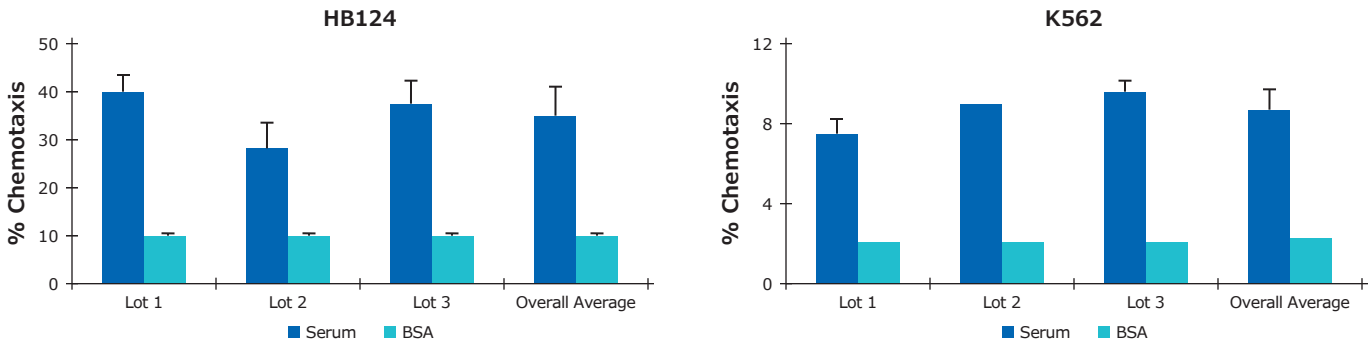

Figure 2. Percent chemotaxis exhibited by HB-124 and K-562 cells in response to 10% serum or 0.2% BSA containing medium as chemoattractant (per lot: n=5, r=24 for HB-124; per lot: n=2, r=24 for K-562 cells, where n is the number of plates and r is the number of wells tested).

2. Chemotaxis assay with adherent cells.

Inter-plate, inter-lot, and intraplate values were obtained with MDA-MB-231 cells.

3. Chemotaxis inhibition assay with adherent cells.

Chemotaxis inhibition experiments were performed to assess the performance of MultiScreen®-MIC plates in drug discovery screening type assays and to assess repeatability of inhibition response.

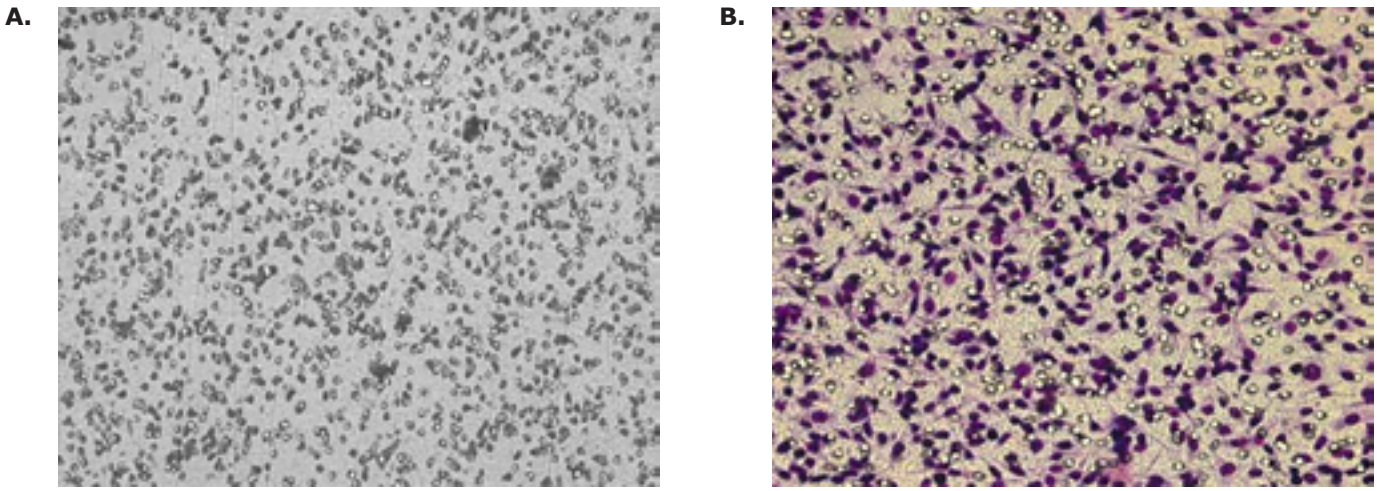

Figure 3. Chemotaxis of highly migratory MDA-MB-231 cells in response to (A) 0.2% BSA containing medium and (B) 10% serum-containing medium as a chemoattractant on 5 µm MultiScreen®-MIC plate. Cells were stained with Hema-3 stain kit.

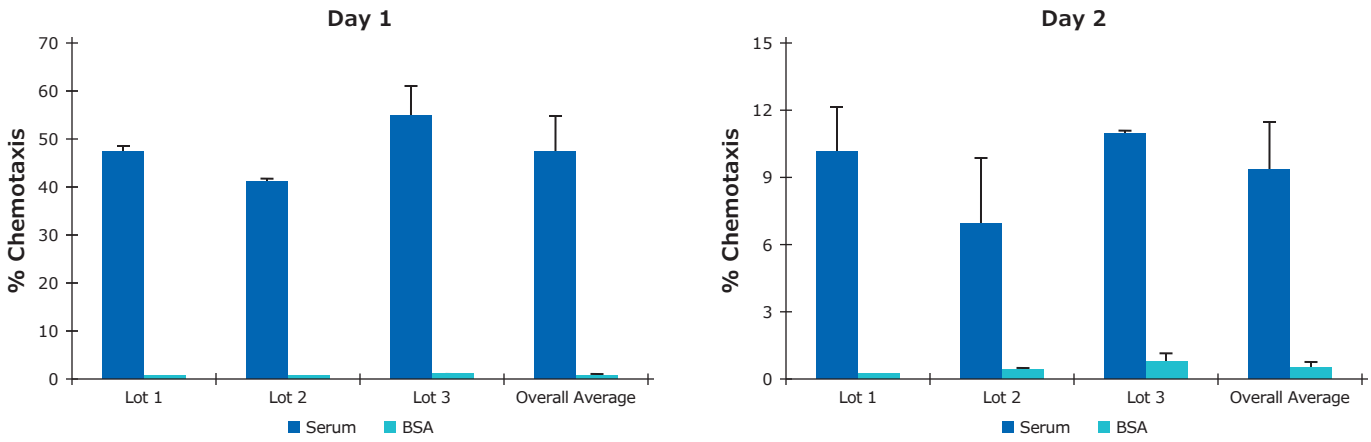

Figure 4. Percent chemotaxis exhibited by MDA-MB-231 cells in response to 10% serum or 0.2% BSA containing medium as chemoattractant (per lot: n=2, r=80 on Day 1; per lot: n=3, r=40 or 56 on Day 2, where n is the number of plates and r is the number of wells tested).

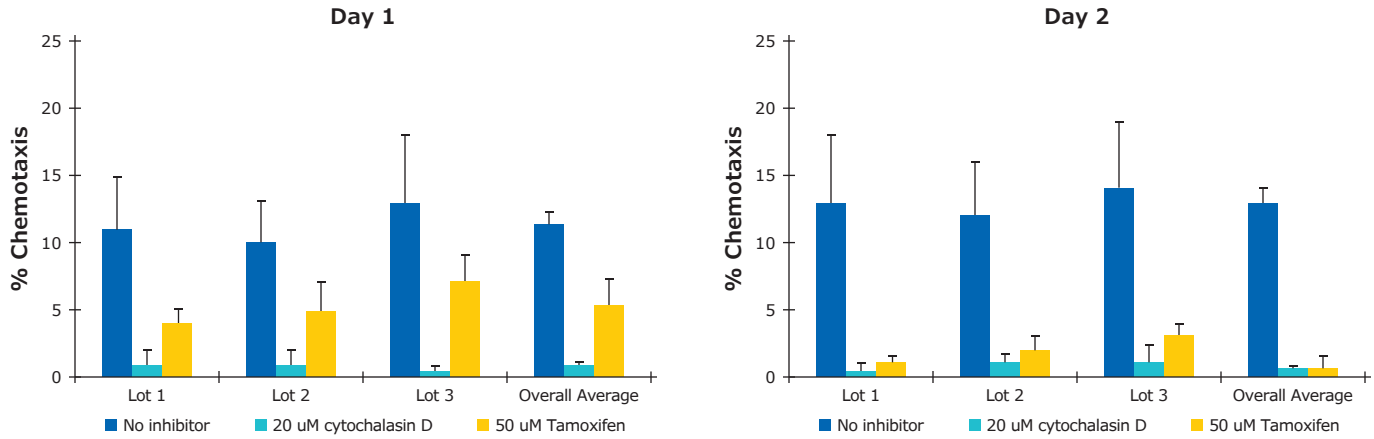

Figure 5. Chemotaxis inhibition profiles of MDA-MB-231 cells on 5 µm MultiScreen®-MIC plates in response to 10% serum or 0.2% BSA serum containing medium as chemoattractant in the presence or absence of inhibitors (per lot: n=1, r=14 per condition tested, on each experimental day, where n is the number of plates and r is the number of wells tested). Average background % chemotaxis values in the absence of inhibitors ranged from 0.3 to 0.4 on day 1 and 0.1 to 0.7 on day 2 indicating robust stimulated chemotaxis response.

Discussion

Percent chemotaxis was assessed with cells in response to 10% serum. HB-124 cells exhibited an overall average chemotaxis of 35% ±6, and data was consistent and reproducible within a range of 15% for the three lots tested. K-562 cells exhibited an overall chemotaxis average of 8.7% ±1, and data was consistent and reproducible within a range of 2% for the three lots tested. MDA-MB-231 exhibited consistent chemotaxis values of 41-55% obtained on Day 1 and 7-11% on Day 2. Interplate and interlot standard deviations were consistent on lots tested on the same experimental day. Intraplate standard deviation values were higher compared to suspension cells and were in the range of 13-29% for assays performed on Day 1 and 2-7% for assays performed on Day 2 for all lots tested. This variability can be attributed to the additional processing steps involved in performing the assay with adherent cells and with the detection method used.

Although consistent and reproducible inhibition results were obtained on lots tested on the same day, assay variability was also observed with HT-1080 cells, including higher values obtained on Day 1 compared to Day 2. These variables should be considered when interpreting assay results across different days.

Chemotaxis inhibition profiles were assessed with cells in response to Cytochalasin D and Tamoxifen inhibitors. Percent inhibition was observed with Cytochalasin D on Day 1 and Day 2 between 93-97%. Variable percent inhibition was observed with Tamoxifen, exhibiting 50-60% on Day 1, and 79-91% on Day 2. These variables should be considered when interpreting assay results across different days.

The results demonstrate the utility of MultiScreen®-MIC plates for chemotaxis and chemotaxis inhibition assays with suspension cells and adherent cells.

General protocol considerations

Chemotaxis assays are complex assays that provide valuable information about cell behavior. General protocol considerations include:

1. Cell type and pore size determination: Smaller pore sizes may be optimal for suspension cells and larger pore sizes more suitable for adherent cells. A comprehensive literature search before performing the assay can provide a starting point for assay parameters.
2. Assay standardization: Standardization experiments can be run to optimize cell density, time of assay, chemoattractant concentrations, and cell to pore ratio to determine optimal assay conditions with appropriate controls.
3. Cell variability: Cell variability is an inherent part of cell-based assays. It is best to interpret results in relation to controls run on the same experimental day for any given assay.

References

1. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, McEwan RN. 1987. A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. Cancer Res. 47(12):3239-3245.
2. Kamath L, Meydani A, Foss F, Kuliopulos A. 2001. Signaling from protease-activated receptor-1 inhibits migration and invasion of breast cancer cells. Cancer Res. 61(15):5933-5940.

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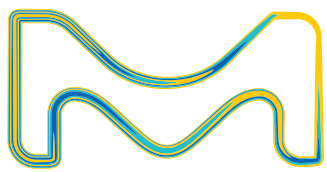

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