Technical Bulletin
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Boyden Chamber Assay with CollOvine™

Collagen is a major component of the basement membrane and tissue scaffolding protein. The ability of tumor cells to invade through a collagen barrier is directly correlated with metastatic potential. The Boyden Chamber assay (**Figure 1**) is one of the most widely accepted cell migration study techniques.

Methods

Cell culture inserts with 8 µm pores in the PET membrane (ThinCert, Greiner Bio-One; 24-well size) were coated with 100 µL of CollOvine, the chambers were dried overnight at 37°C. HT-1080 cells (ATCC CCL-121, human fibrosarcoma cell line) or negative control cells (HEK-293) were deprived in serum-free culture medium the night before the migration experiment. On the day of the experiment, 700 µL of complete media (*i.e.*, with serum) was added to the bottom chamber, and 200 µL of cell suspension (6.8 x 10^5 cells/mL) in serum-free media was added to the top chamber. The chambers were incubated overnight at 37°C and 5% CO_2. The next day, the culture media in the lower chamber were replaced with 450 µL serum-free media and 8 µM Calcein-AM and incubated for 45 minutes at 37°C and 5% CO_2. The inserts were then transferred to fresh 24-well plates containing 500 µL 0.2X Trypsin-EDTA per well and incubated for 10 minutes at 37°C and 5% CO_2, with agitation. Two hundred and fifty microliters of each detached cell suspension were then transferred to 96-well plates for quantification with a fluorescence plate reader at 485 nm excitation and 520 nm emission.

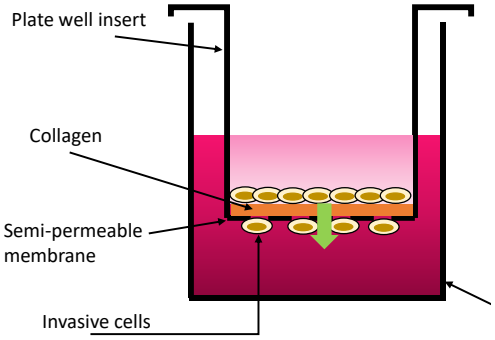

Figure 1. Schematic of a Boyden Chamber Assay.

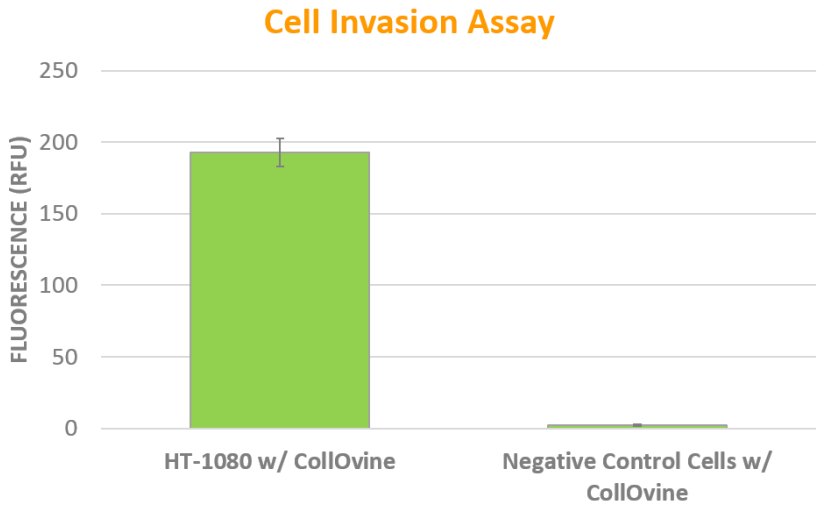

Figure 2. Fluorescence of HT-1080 cells migrated through a CollOvine-coated Boyden chamber.

Results

Figure 2 shows that the HT-1080 was able to invade through a CollOvine-coated membrane, while the negative control cells (HEK-293) was not able to invade through, as expected.