Video Article

An *In vitro* FluoroBlok Tumor Invasion Assay

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Abstract

The hallmark of metastatic cells is their ability to invade through the basement membrane and migrate to other parts of the body. Cells must be able to both secrete proteases that break down the basement membrane as well as migrate in order to be invasive. BD BioCoat Tumor Invasion System provides cells with conditions that allow assessment of their invasive property *in vitro*[1,2]. It consists of a BD Falcon FluoroBlok 24-Multiwell Insert Plate with an 8.0 micron pore size PET membrane that has been uniformly coated with BD Matrigel Matrix. This uniform layer of BD Matrigel Matrix serves as a reconstituted basement membrane *in vitro* providing a true barrier to non-invasive cells while presenting an appropriate protein structure to study invasion. The coating process occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. In contrast, invasive cells are able to detach themselves from and migrate through the coated membrane. Quantitation of cell invasion can be achieved by either pre- or post-cell invasion labeling with a fluorescent dye such as $DiIC_{12}(3)$ or calcein AM, respectively, and measuring the fluorescence of invading cells. Since the BD FluoroBlok membrane effectively blocks the passage of light from 490-700 nm at >99% efficiency, fluorescently-labeled cells that have not invaded are not detected by a bottom-reading fluorescence plate reader. However, cells that have invaded to the underside of the membrane are no longer shielded from the light source and are detected with the respective plate reader. This video demonstrates an endpoint cell invasion assay, using calcein AM to detect invaded cells.

Video Link

The video component of this article can be found at https://www.jove.com/video/1475/

Protocol

Post-labeling and measurement using BD calcein AM Fluorescent Dye

Cells are labeled for quantitation after they have invaded through the BD Matrigel Matrix and passed through the BD FluoroBlok membrane. As a result, only endpoint measurement of cell invasion may be obtained.

1. Grow cells to ~80% confluence.
2. Prepare and rehydrate the insert system.
    1. Remove the package from -20°C storage and allow it to come to room temperature.
    2. Open the foil package and add 500 µL warm (37°C) media to the interior of the insert wells. Allow the plate to rehydrate for 2 hours at 37°C, 5% CO_2.
    Note: It is not necessary to rehydrate the uncoated BD Falcon FluoroBlok 24-Multiwell Insert System that will be used as a cell migration control.
    3. After rehydration, carefully remove the medium from the insert wells without disturbing the layer of BD Matrigel Matrix on the membrane. The system is now ready to use.
3. Prepare cell suspensions by trypsinizing cell monolayers and resuspending the cells in serum-free DMEM at 5×10^4 cells/mL.
Note: If you are using a different cell type you need to determine the optimal seeding density. To determine the optimal seeding density for your cell type on a porous growth surface, use a range of seeding densities (cells/cm^2) that brackets the seeding density used on nonporous surfaces (i.e. flasks, dishes and plates). For example, if you currently seed at 2.5×10^5 cells/cm^2, seed at various cell concentrations between 5×10^4 and 5×10^5 cells/cm^2 to determine the optimal initial seeding density.
4. Add 500 µL of cell suspension (2.5×10^4 cells) to the apical chambers.
5. Add 750 µL of chemoattractant (5% FBS in DMEM) to each of the basal chambers, using the sample ports for access.
6. Incubate the BD BioCoat Tumor Invasion System and the uncoated BD Falcon FluoroBlok 24-Multiwell Insert Plate for 20-22 hours at 37°C, 5% CO_2 atmosphere.
7. Following incubation, carefully remove medium from the apical chambers. This can be accomplished by flicking the contents into a waste container. Do not touch the bottom surface of the insert system.

8. Transfer the insert system into a second 24-well plate containing 500 µL/well of 4 µg/mL Calcein AM in HBSS. Incubate for 1 hour at 37°C, 5% CO_2. The Calcein AM solution is not removed from the lower chamber before reading fluorescence because it is a nonfluorescent vital dye that is converted into green fluorescent calcein by cytosolic esterases.
9. Fluorescence of invaded cells is read at wavelengths of 494/517 nm (Ex/Em) on a bottom-reading fluorescent plate reader. The gain setting may need to be determined empirically, but a midpoint gain should be a sufficient starting point. A gain setting that is too high may lead to saturation of the detector with highly fluorescent sample; this may prevent the acquisition of meaningful results. Use of autogain (if supported on your reader) is not recommended. An inverted fluorescence microscope can be used to verify your results; it is especially helpful to do this the first time you run this assay.
   Note: It is of utmost importance that the Insert Systems are read using the correct plate map. For information on loading plate maps see BD Technical Bulletin No. 436 on www.bdbiosciences.com or contact BD Technical Support (labware@bd.com). Proper plate orientation is with well A1 at the top left corner and the BD Flacon logo oriented to the right as the plate is inserted into the reader.

Data Reduction

Data is expressed as in the following equation:

$$\% \text{ Invasion} = \frac{\text{Mean RFU of cells invaded through BD Matrigel™ Matrix coated membrane towards che}}{\text{Mean RFU of cells migrated through uncoated BD FluoroBlok membrane towards chem}}$$

Background may be subtracted prior to the calculation of percent cell invasion
RFU = relative fluorescent units.

Disclosures

The authors are employees of BD Biosciences that produces reagents and tools used in this article.

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