Frequently Asked Questions

FAQ: Millicell® µ-Migration Assay Kit (MMA205)

Visualizing

1. What equipment and software do I need to track migrating cells?	You will need a phase contrast microscope or fluorescence inverted microscope, heating stage, time-lapse video equipment (CCD camera, video camera, acquisition software), motorized stage and autofocus (x, y, z) and the NIH Image J plug-in software. Instructions for free download of this software are available at www.millipore.com/umigration).
2. Can I conduct the migration assay with fluorescently stained cells?	Yes. Fluorescent staining is compatible with the migration kit. The cells are pre-stained before loading into the slide.
3. Can I use a normal digital camera connected to a microscope to take images manually at different time points (e.g. if the microscope doesn't have video equipment)? If so, can this data be opened in the Image J plug-in software and analyzed?	Yes. To analyze the pictures with the Image J software, just follow these steps: 1. If taking pictures manually at different time points, be sure to take them with the slide always at the same position on the microscope. 2. Save the image files in order. 3. Import the files into Image J—the software will organize the images in order. 4. Analyze the data using Image J software.
4. Can I take images of assays running concurrently in different chambers on the same slide?	This is easily done with a microscope that has a computer-controlled movable stage. If the microscope doesn't have a computer-controlled movable stage, it is very difficult to manually take all the pictures/videos from 3 chambers at different time points (or at the same time) since the registration (original point) is lost for each of the different chambers. The migration of cells is very small, usually several microns/hour. If the photos are taken manually in a slightly different area, the trace of the cells is not accurate. In this case, we recommend running one chamber at a time in individual assays.
5. If multiple assays are run on a slide and images are captured for all of them, can the Image J software identify which chamber each photo derives from at different time points?	No, Image J software cannot sort the photos taken of simultaneous assays. You will need to sort the pictures before loading into Image J.
6. If I don't have a heating stage, is it possible to keep the slide in the incubator during the assay and take it out to capture images at different time intervals?	The migration of cells is very small, usually several microns/hour. To get accurate data, you must observe the same exact area at different time points. If you can engineer a method to ensure that you are taking images of the exact same area each time you remove and replace the slide, then it is possible to use an incubator instead of a heating stage. However, remember that you will be taking images every 10–15 minutes for 12 hours, so a heating stage is recommended.
7. How can I correctly track the recommended 20 to 50 cells which are necessary to obtain representative data in one image/frame?	Please refer to the Image J plug-in software document available at www.millipore.com/umigration.
8. What the effective surface area of the viewing area?	The effective viewing area depends on the objective magnitude of the microscope used. The appropriate magnitude should cover the entire area encompassing the cells, the source, and the paths of the cells migrating towards/against the source (1 mm, y-direction).

Coating and other slide handling

1. I am using fibronectin and I never let it dry out before seeding cells. However, the protocol suggests drying the slide out completely. Can I seed the cells immediately after coating like I normally do when culturing my cells?	No. As this is a microfluidic device, it is critical to ensure complete evaporation of fluid from the slide prior to cell loading so that air bubbles do not get trapped in the channels.
2. Do I need to worry about CO_2 or O_2 gas exchange?	For CO_2 dependent cell lines, CO_2 gas exchange is necessary during real-time imaging. The slide's plastic is gas-permeable thus allowing for some gas exchange to occur. A CO_2 gas exchanger is optimal.
3. Can the conditioning or incubation of the chambers be shortened at all?	No. As this is a microfluidic device, it is critical to ensure complete evaporation of all fluid from the slide prior to cell loading so that air bubbles do not get trapped in the channels.
4. I lost all of my cells when I changed medium to serum-free medium. How can I prevent this from happening next time?	The cells likely did not attach well. Following the suggested coating procedure should improve cell attachment and prevent this cell loss from occurring again during the media exchange step.
5. Can I use another extracellular matrix (ECM) to coat the slides?	Yes, it is possible to coat the slide using other ECMs. We suggest that you try several surfaces to choose the appropriate concentration of coating according to different cell lines. For example, we suggest coating the slide with 30 µL fibronectin, following the coating procedure outlined in the user guide.

Additional applications

1. Can I use this migration assay in addition to a Boyden chamber?	Yes. The Millicell µ-migration kit provides complementary benefits to using a Boyden chamber, including: 1. Ability to determine that cell migration is due to directional chemotaxis response instead of activation of cell movement. • The limitation of Boyden chambers is that cells do not encounter a chemogradient, but rather a sharp increase of chemoattractants. Therefore, the migration event might be a result of active cell movement. Think of it as cells bouncing around instead of moving towards a target. 2. Ability to determine that cells are responding to soluble factors in an invasion assay. • In invasion assays, cells digest extracellular matrix components (or other artificial barrier) in order to migrate through them. Using the Millicell µ-migration assay can help determine the soluble factor that triggers migration. 3. Ability to test chemo-repellents (factors which cells try to avoid). This is almost impossible to test in a Boyden chamber.
2. I would like to try a migration assay with fibroblasts but I would like to use activated macrophages or neutrophils as chemoattractants. Is it possible to add these cells instead of a chemoattractant?	We have not tested this application. To ensure macrophages reach the observation area when they are added as the chemoattractant, add less volume than the suggested chemoattractant volume (18 µL).

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