

Oris™ Cell Migration Assembly Kit ! FLEX

Product No.: CMAUFL4

96-well Assay for Investigating
Cell Migration and Cell Invasion of Adherent Cell Lines

PROTOCOL & INSTRUCTIONS

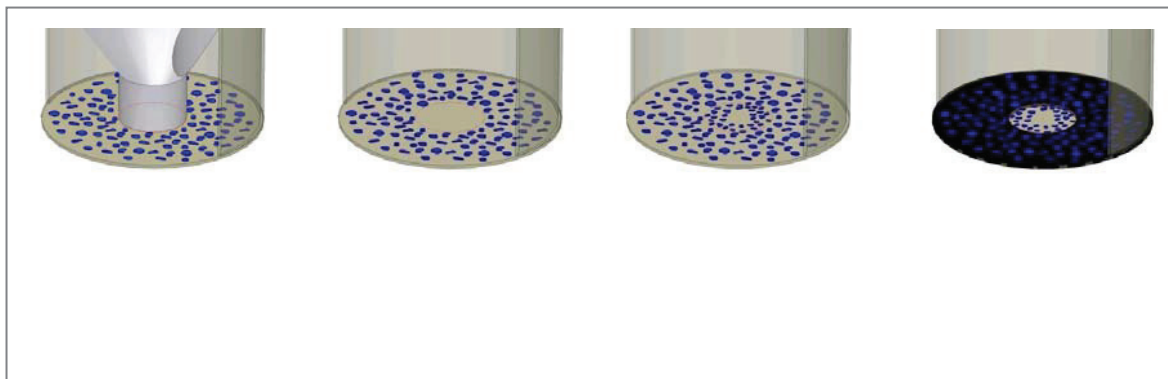
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ORIS" CELL MIGRATION ASSEMBLY KIT ! FLEX

The Oris" Cell Migration Assembly Kit - FLEX has been designed for use with adherent cell cultures. This assay has been successfully used with 3T3-Swiss albino, HT-1080, HCEC, and MCF10A cell lines.

Using the Oris" Cell Migration Assembly Kit - FLEX offers the following features & benefits:

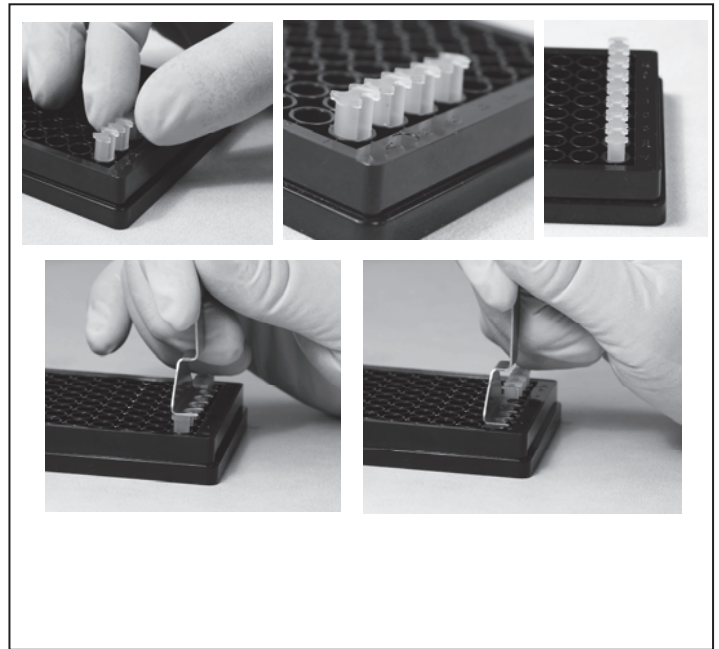
- ! Perform partial plate experiments without compromising sterility or integrity of the plate.
- ! Analyze cells treated with multiple fluorescent probes, labels or stains by using a microplate reader, microscope, or digital imaging system.
- ! Coat any ECM or BME on the plate to create a 2-D or 3-D environment for cell migration or cell invasion assays.
- ! Design kinetic or endpoint assays without the use of special instrumentation.
- Achieve lower well-to-well CV#s with the unique Oris" assay design than with scratch assays.
- ! Track changes in cell movement and morphology as cell migration or invasion progresses.
- ! Observe cells directly without interference from cell culture inserts or transmembrane devices.



: If desired, coat the bottom of the wells with an ECM layer (collagen, fibronectin, laminin, etc.) and allow the ECM to dry prior to populating the plate with the Oris" Cell Seeding Stoppers.

1. Under sterile conditions, populate the 96-well plate with the desired number of Oris" Cell Seeding Stoppers (provided in 4-stopper strips):

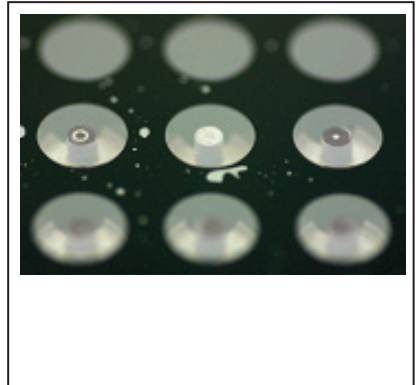
- Vertically position the tip ends of two, 4-stopper strips into one full column of 8 wells at a time (Figure 2A).
- Gently press down on the strip backbone to partially insert the stoppers halfway into the well (Figure 2B).
- When both stopper strips have been partially inserted in 1 column, ensure that the position of the stoppers is vertical with respect to the well wall, making any necessary adjustments (Figure 2C).
- Using the Oris" Stopper Tool, firmly press down on the strip backbone to fully insert the stoppers into each well (Figure 2D and 2E). Repeat for the remaining columns that you require for your experiment.



It is extremely important to ensure that the stoppers are inserted perpendicular to the well bottom and are fully engaged with the well bottom. Failure to do so will increase the CV of your data set. If you require data sets with low CVs [potential for $\leq 12\%$], the pre-populated Oris" Cell Migration Assay kit (\$CMA1.101) is recommended.

Once the sterile pouch of Oris" Cell Seeding Stoppers has been opened, handle the stoppers aseptically. Any unused stoppers can be kept in a sterile environment (i.e., laminar flow hood/UV light). Do not autoclave the stoppers.

2. Visually inspect the underside of the populated 96-well plate to ensure that the Oris" Cell Seeding Stoppers are firmly sealed against the bottom of the plate. To inspect the stoppers, turn the plate over and examine the stoppers for sealing (see Figure 3). If incomplete sealing is observed, return the plate to the upright position and use a sterile instrument to gently push the stopper back into the well until sealing is observed.

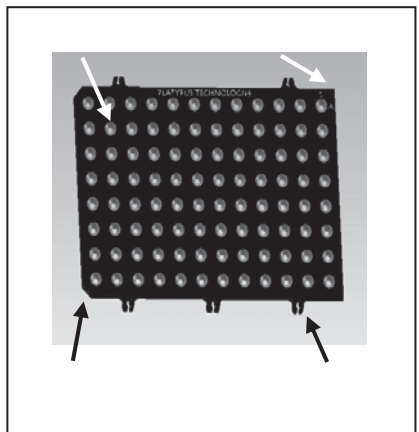


the sealing of the stoppers can be most easily observed if the plate is tipped at an angle and viewed under indirect light to reveal the bullseye pattern at the bottom of each well.

3. Apply the Oris" Detection Mask to the bottom of the 96-well plate if microplate reader data is being collected. The Detection Mask is not necessary if collecting imaging data.

: In order to prevent splashing of well contents, familiarize yourself with the attachment and removal of the Detection Mask before any liquids are placed in the wells.

- Orient the chamfered corners of the mask with those of the 96-well plate, ensuring that the A1 corner of the mask is aligned with the A1 well of the plate (see Figure 4).
- Align the holes in the attachment lugs with the bosses on the bottom of the 96-well plate.
- Gently press the mask until it is flush with the bottom of the 96-well plate.



It may be necessary to wash the mask with ethanol to remove dust and debris since the mask is sterile. The mask may be applied at any point during the assay. For kinetic assays, it is often most convenient to apply the mask at the beginning of the assay before any liquids are placed in the well. For endpoint assays, using fixed and stained cells, it is often most convenient to apply the mask just before reading assay results.

