tebu-bio

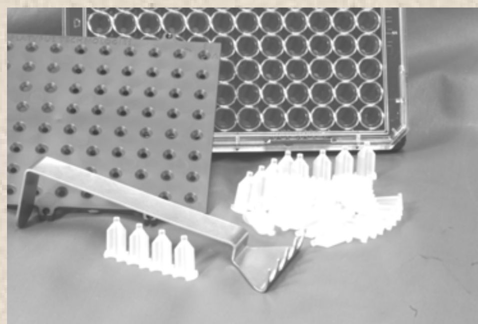
# Universal Cell Migration Assembly Kit

Product No.: CMAU101 & CMAU505

96-well Assay for Investigating
Cell Migration, Cell Invasion and 2-D Closure
of Adherent Cell Lines

## Protocol & Instructions

Patent Pending

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# Oris™ Universal Cell Migration Assembly Kit

## I. INTRODUCTION

The Oris™ Universal Cell Migration Assembly Kit is a reproducible, sensitive, and flexible assay that can be used to monitor cell migration, cell invasion and 2-D closure. The Oris™ Universal Cell Migration Assembly Kit now gives researchers more control over designing a cell migration assay. Each Oris™ Cell Migration Assembly kit contains the Oris™ Cell Seeding Stoppers for creating a detection zone at the center of each well in a 96-well plate. Since the stoppers are not pre-inserted into the wells, researchers have two options; coat the plate with an extracellular matrix (ECM) component or use the coated plate provided to design an assay. Researchers may also apply 3-dimensional overlays in each well to watch how cells invade and respond to various compounds (chemokinesis). Each kit is supplied with a 96-well, black-clear bottom plate, an Oris™ Detection Mask, an Oris™ Stopper Tool, and Oris™ Cell Seeding Stoppers.

The Oris™ Universal Cell Migration Assembly Kit has been designed for use with adherent cell cultures. This assay has been successfully used with fibroblast (3T3-Swiss albino), fibrosarcoma (HT-1080), and endothelial (HCEC and MCF10A) cell lines.

The Oris™ Universal Cell Migration Assembly Kit offers the following benefits:

- **Membrane-free, Cell Migration or Invasion** - no cumbersome cell culture inserts to manipulate or limit cellular movement; there is no Transwell® membrane insert to block live images of cell movement.
- **Creative Assay Design** - coat any ECM or BME on the plate to create a 2-D or 3-D environment for cell migration, cell invasion or 2-D closure assays.
- **Preserve Cell Morphology** - the Oris™ assay provides a more native environment since cells do not have to penetrate through a polycarbonate membrane; cells can move across a tissue culture treated surface or within a user-applied ECM.
- **Real-time Monitoring** - changes in cell structure during movement can be monitored in real-time with a microscope, digital imaging system or fluorescence plate reader.
- **Reproducible Results** - 2-D closure assays using the Oris™ Cell Seeding Stoppers have better reproducibility than wound healing or scratch assays.
- **Versatile Detection Modes** - analyze cells using multiple fluorescent probes, labels or colorimetric stains in the same well.
- **Flexible Assay Formats** - design kinetic or endpoint assays without the use of special instrumentation.

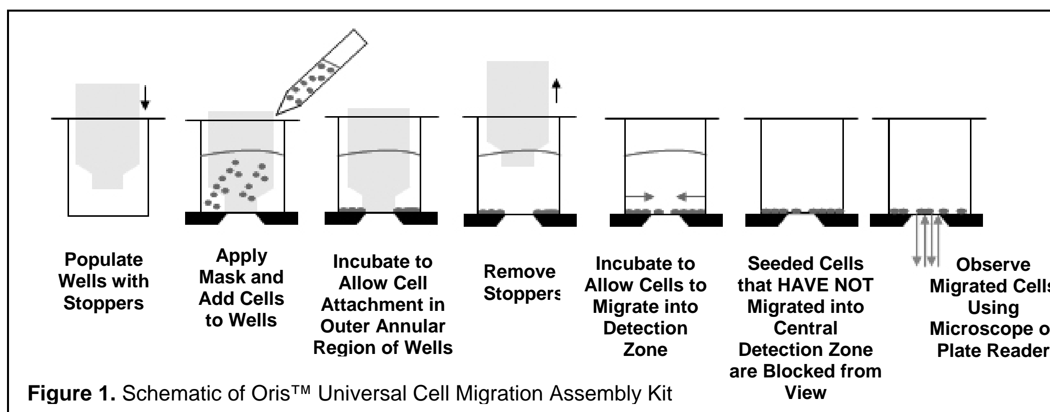


**Figure 1.** Schematic of Oris™ Universal Cell Migration Assembly Kit

## II. ORIS™ PLATE DIMENSIONS (per well)

| | |
|---|---|
| Diameter of Well | 6.5 mm |
| Diameter of Stopper Space (Detection Zone) | 2 mm |
| Suggested Media Volume per Well (populated with Stoppers) | 100 µl |
| Effective Area of Outer Annular Region (seeding region) per Well | 30.03 mm$^2$ |
| Effective Area of Central Detection Zone per Well | 3.14 mm$^2$ |
| Storage Conditions | Ambient |

**Important**: Read Instructions Before Performing any Oris™ Assay.

## III. MATERIALS PROVIDED

**Product No.: CMAU101**
Oris™-compatible, 96-well (black, clear bottom) Plate, 1
Oris™ Cell Seeding Stoppers, 96
Oris™ Detection Mask, 1
Oris™ Stopper Tool, 1

**Product No.: CMAU505**
Oris™-compatible, 96-well (black, clear bottom) Plates, 5
Oris™ Cell Seeding Stoppers, 5 x 96
Oris™ Detection Mask, 1
Oris™ Stopper Tool, 1

## IV. MATERIALS REQUIRED

- Biological Cells
- Cell Culture Medium
- Sterile PBS
- Sterile Pipette Tips and Pipette or Multi-Channel Pipette
- Trypsin or Non-Enzymatic Cell Removal Reagent or Scraper
- Inverted Microscope (optional)
- Fluorescence Microplate Reader (optional)
- Cell Labeling Fluorescent Agent (eg., CellTracker™ Green*, Calcein AM) - *required if performing assay readout via plate reader.* *a product of Molecular Probes/Invitrogen
- Extracellular Matrix (ECM) or Basement membrane Extract (BME) for creating a 3-D assay (optional)

## V. ORIS™ UNIVERSAL CELL MIGRATION ASSEMBLY KIT PROTOCOL

The following steps should be performed in a biological hood using aseptic technique to prevent contamination.

1. Under sterile conditions, populate the 96-well plate with Oris™ Cell Seeding Stoppers:

   - 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 all remaining columns.

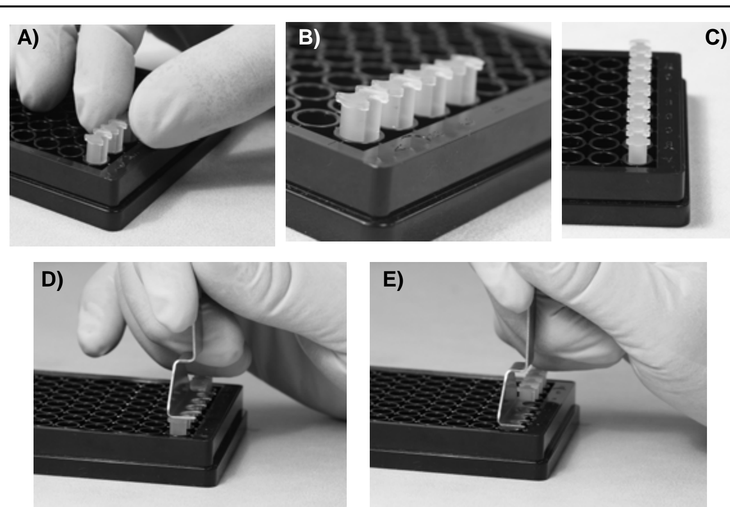


**Figure 2.** Stopper Insertion Process. A) Placement of Stoppers into Wells, B) Close-up of Stoppers Partially Inserted into Wells, C) Proper Placement of Stoppers, D) Pressing of Stoppers into Wells, and E) Fully Inserted Stoppers

**NOTE:** It is extremely important to ensure that the stoppers are inserted perpendicular to the well bottom and 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 %], it is recommended to use the pre-populated Oris™ Cell Migration Assay kit (#CMA1.101).

**Optional**: If desired, coat the bottom of the wells with an Extracellular Matrix (ECM) component (collagen, fibronectin, laminin, etc.) and allow the ECM to dry prior to populating the plate with the Oris™ Cell Seeding 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.

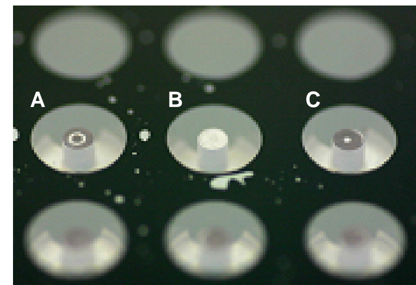


**Figure 3.** Stoppers that are A) Partially Sealed, B) Unsealed, & C) Completely Sealed

**NOTE:** the sealing of the stoppers can be most easily observed if the plate is tipped at an angle and viewed under indirect light looking for the bullseye pattern at the bottom of each well (see Figure 3).

3. Apply the Oris™ Detection Mask to the bottom of the 96-well plate.

   **First Time Users**: 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 (see Figure 4) is aligned with the A1 well of the plate.
   - 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.

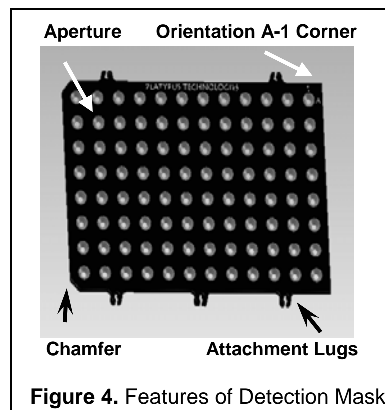


**Figure 4.** Features of Detection Mask

**NOTE:** It may be necessary to wash the mask with ethanol to remove dust and debris since the mask is **not** 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.

4. If performing a kinetic analysis of cell migration, pre-stain cells to be seeded with a fluorescent stain now.

5. Collect cells and prepare a suspension that is 10-fold greater in density than the optimal seeding concentration.

   **First Time Users**: The optimum seeding density of cells must be determined as an integral part of the design of the cell migration assay. Please see Appendix I for a discussion of this process.

6. Pipette 100 µl of suspended cells into each test well through one of the side ports of the Cell Seeding Stopper.

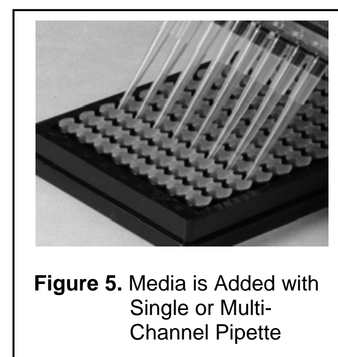

**Figure 5.** Media is Added with Single or Multi-Channel Pipette

**NOTE:** For best results, add or extract media by placing the pipette tip along the wall of the well (see Figure 5). Care should be taken not to disturb the Cell Seeding Stopper when introducing the pipette tip into the well. A gel loading tip may be useful.

7. **IMPORTANT**: Gently tap the plate on your work surface to evenly distribute well contents (extreme tapping may result in splashing of well contents and lead to contamination).

8. Incubate the seeded plate containing the Oris™ Cell Seeding Stoppers in a humidified chamber (37°C, 5% $CO_2$) for 4 to 16 hours (cell line dependent) to permit cell attachment.

9. Remove plate from incubator.

10. Designate several 'reference' wells in which the stoppers will remain in place until results are read (t=0 pre-migration controls).

11. Using the Oris™ Stopper Tool, remove all other stoppers (see Figure 6).
    - Secure the 96-well plate by holding it firmly against the deck of your work space. Slide the tines of the stopper tool under the backbone of the stopper strip, keeping the underside of the tool flush with the top surface of the plate.
    - Lift the stopper tool ***vertically*** to gently remove the stopper.

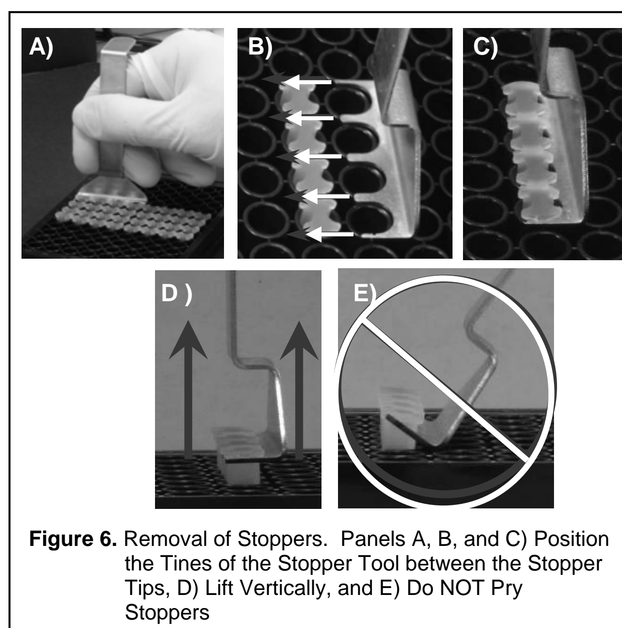


**Figure 6.** Removal of Stoppers. Panels A, B, and C) Position the Tines of the Stopper Tool between the Stopper Tips, D) Lift Vertically, and E) Do NOT Pry Stoppers

**NOTE: DO NOT** use the stopper tool as a lever to pry the stoppers from the well (see Figure 6E), as doing so may cause displacement of seeded cells and may distort the detection zone area.

12. Remove media with a pipette and ***gently*** wash wells with 100 µl PBS (or media) to remove any unattached cells. Do not aspirate using an in-house vacuum.

    **Optional**: If the plate was originally coated with an ECM (in Step 1), an overlay of ECM may be introduced in the wells to facilitate a 3-Dimensional invasion assay. Optimization of experimental conditions will be required to establish invasion conditions for a given cell line.

13. Add 100 µl of fresh culture media to each well.

14. Incubate plate in a humidified chamber (37°C, 5% $CO_2$) to permit cell migration. Incubation time will vary depending upon cell type and experimental design.

15. If performing an endpoint analysis of cell migration, apply stain.

**NOTE:** Oris™ Cell Seeding Stoppers are for single use only; Platypus can not guarantee the integrity of the stopper material after a second sterilization procedure.

## VI. DATA ACQUISITION

The readout of the Oris™ Universal Cell Migration Assembly Kit can be conducted at any time, allowing the user to perform a kinetic assay or an endpoint assay. The Oris™ Universal Cell Migration Assembly Kit is designed to be used with any commercially available stain or labeling technique. The readout can be performed by microscopic examination or by plate reader.

Microscopic Analysis

- Cell counting or image capture / analysis (using software, such as Image J freeware, available from NIH).
- Sample Data using a colorimetric stain is shown in Figure 7. Wells were seeded with 50,000 HT-1080 cells (i.e., 100 µl of $5x10^5$ cells/mL) and incubated for 4 hours. The stoppers were removed from test wells, but remained in place in the pre-migration reference wells until the time of the assay readout. The seeded plate was incubated in a humidified chamber for 24 hours to permit cell migration. Stoppers were removed from the reference wells and all cells were fixed and treated with Wright-Giemsa stain. Images were captured using bright field microscopy and then imported to Image J software for analysis using thresholding. The images below, captured without a detection mask in place, illustrate representative data from pre-migration (t=0 hrs) and post-migration (t=24 hrs) wells. The graph depicts the average pixel number in the detection zones for each condition.

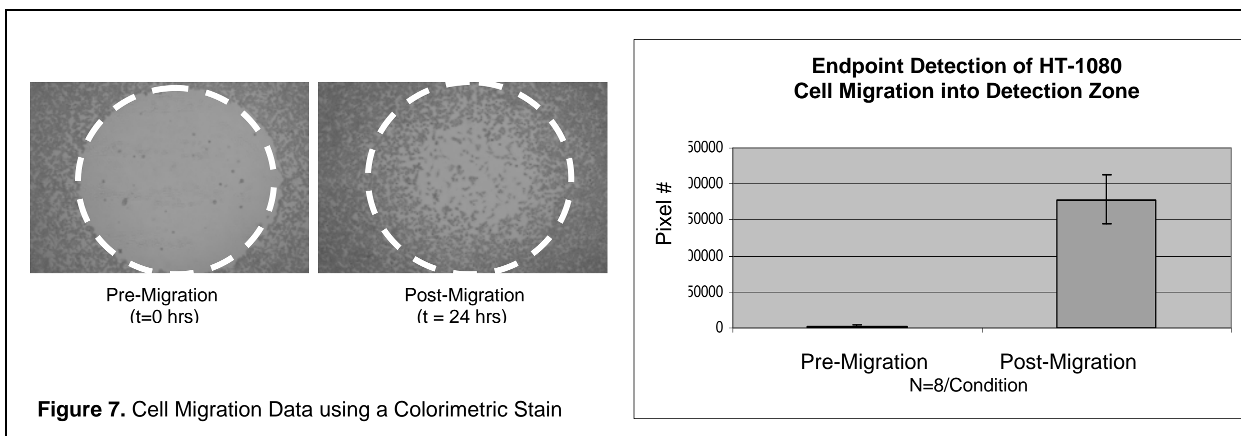


**Figure 7.** Cell Migration Data using a Colorimetric Stain

Plate Reader Analysis

- Setup on individual plate readers varies according to make and model. Consult your user manual for proper operation.
- The plate reader MUST be set to use the bottom probe read.
- Sample Data using a fluorescent stain is shown in Figure 8. Wells were seeded with 50,000 HT-1080 cells (i.e., 100 µl of $5x10^5$ cells/mL) and incubated for 4 hours. The stoppers were removed from test wells, but remained in place in the pre-migration reference wells until the time of the assay readout. Cells were fluorescently stained with CellTracker™ Green. The seeded plate was incubated in a humidified chamber for 28 hours and at various time points the fluorescence signals in the detection zones were measured using a plate reader. The images below, captured without a detection mask in place, illustrate representative data from pre-migration (t=0 hrs) and post-migration (t = 21 hrs) wells. The graph depicts a real-time analysis of cell migration that was prepared by transposing the fluorescent signal into cell numbers by employing a standard curve and a 5-Parameter Logistic-fit Equation.

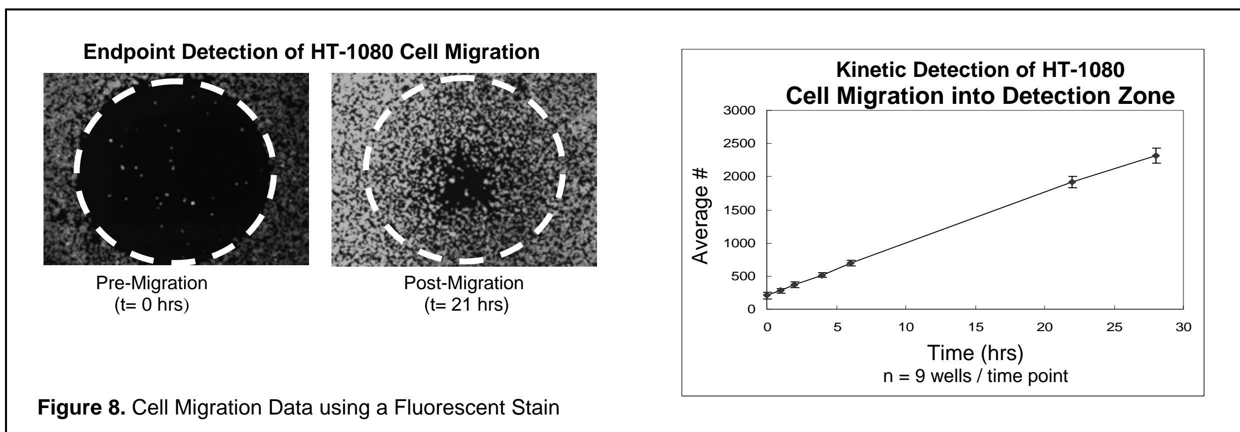


**Figure 8.** Cell Migration Data using a Fluorescent Stain

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CellTracker™ Green is a trademark of Invitrogen Corporation.

Transwell® is a registered trademark of Corning, Inc.

## VII. ORDERING INFORMATION

| Product No. | Product Description | Package Size |
|---|---|---|
| CMAU101 | Oris™ Universal Cell Migration Assembly Kit, 1-pack:<br>Oris™-compatible, 96-well plate (black, clear bottom), 1<br>Oris™ Cell Seeding Stoppers, 96<br>Oris™ Detection Mask, 1<br>Oris™ Stopper Tool, 1 | 1-pack |
| CMAU505 | Oris™ Universal Cell Migration Assembly Kit, 5-pack:<br>Oris™-compatible, 96-well plates (black, clear bottom), 5<br>Oris™ Cell Seeding Stoppers, 5 x 96<br>Oris™ Detection Mask, 1<br>Oris™ Stopper Tool, 1 | 5-pack |
| CMAUCC1 | Oris™ Cell Migration Assembly Kit - Collagen I Coated, 1-pack:<br>Oris™ Collagen I Coated, 96-well plate (black, clear bottom), 1<br>Oris™ Cell Seeding Stoppers, 96<br>Oris™ Detection Mask, 1<br>Oris™ Stopper Tool, 1 | 1-pack |
| CMAUCC5 | Oris™ Cell Migration Assembly Kit - Collagen I Coated, 5-pack:<br>Oris™ Collagen I Coated, 96-well plates (black, clear bottom), 5<br>Oris™ Cell Seeding Stoppers, 5 x 96<br>Oris™ Detection Mask, 1<br>Oris™ Stopper Tool, 1 | 5-pack |

To place an order, visit the Platypus Technologies website at: www.platypustech.com/order_main.html.
For technical assistance, contact Technical Support at (866) 296-4455 or techsupport@platypustech.com.

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PLATYPUS shall not be liable for injury or damages resulting from the use or misuse of any of its products.

## APPENDIX I: Determining Optimal Cell Seeding Concentration

This appendix is intended to assist in determining the cell seeding density needed to achieve confluency of your cell line when using the Oris™ Universal Cell Migration Assembly Kit. To that end, several dilutions of cell suspensions will be investigated.

**NOTE:** The Oris™ Detection Mask ***MUST*** be removed from the 96-well plate prior to the start of the following steps:

1. Collect cells by trypsinization or by non-enzymatic means (such as mechanical scraping) and calculate total number of cells.
2. Pellet cells by centrifugation and resuspend to a final concentration of 500,000 cells/mL in culture media.
3. Populate test wells with Oris™ Cell Seeding Stoppers (see Steps 1 – 2 of the Protocol).
4. Seed 100 µl of cells, at 2-fold serial dilutions, in the 96-well plate starting at 50,000 cells/well (a suggested starting amount), as shown below. Keep in mind that the cell seeding area of the well with the stopper in place is ~ 0.3 $cm^2$ and based on the typical seeding density of your cells, you can infer the appropriate cell number for your first serial dilution.

| Column | 1 | 2 | 3 |
|---|---|---|---|
| Cells / well | 50,000 | 25,000 | 12,500 |
| Number of wells | 6 | 6 | 6 |

5. Incubate the plate in a humidified chamber (37°C, 5% $CO_2$) for 16 hours with cell seeding stoppers in place.
6. Following cell attachment, remove the Oris™ Cell Seeding Stoppers from each well (see Figure 6) and ***gently*** wash the wells with PBS to remove non-adhered cells.
   - Secure the 96-well plate by holding it firmly against the deck of your work space. Slide the tines of the stopper tool under the backbone of the stopper strip, keeping the underside of the tool flush with the top surface of the plate.
   - Lift the stopper tool ***vertically*** to gently remove the stopper. Do not use the tool as a lever to pry the stoppers from the well as doing so may cause displacement of the seeded cells.
7. Use a microscope to visually inspect the cells and determine the cell seeding concentration that yields a confluent layer.

**NOTE:** If you plan to obtain the results of the Oris™ Universal Cell Migration Assembly Kit via colorimetric or microscopic analysis, you have successfully determined the optimal cell seeding concentration for your cell line. Proceed to Step 5 of the Oris™ Universal Cell Migration Assembly Kit Protocol. If you plan to obtain the results of the Oris™ Universal Cell Migration Assembly Kit via a fluorescence plate reader, proceed with the following steps to optimize your plate reader settings.

8. The Oris™ Universal Cell Migration Assembly Kit has been designed to work with all types of fluorescence stains and staining techniques. The precise method for staining cells with fluorescence stains varies according to the nature of the individual stain. Please consult the manufacturer of your fluorescence stain for specific considerations.

   **First Time Users**: For a guide to using Calcein AM, see below:
   a) Aspirate media from wells & wash wells with PBS or media.
   b) Add 100 µl of Calcein AM to each well at an appropriate concentration [for a fully-seeded 96-well plate, combine 5 µl of reconstituted Calcein AM (1mg/mL in dry DMSO) with 10 mL of serum-free media or 1x PBS].
   c) Incubate plate at 37°C for 20 minutes.
   d) Remove plate from incubator.
   e) Remove staining solution. Do not aspirate using an in-house vacuum.
   f) Fix cells, or to prevent drying, add 100 µl of 1x PBS to each well.

9. Apply the Oris™ Detection Mask to the plate.
10. Using the bottom probe of a fluorescence plate reader, obtain the total output from each well (adjust the gain settings to achieve optimal dynamic range). To determine optimal dynamic range, consider the following factors:
    a) The gain setting that permits detection of the lowest concentration of cells.
    b) The gain setting that permits discrimination between cell numbers at higher densities.

**NOTE**: When using a plate reader to analyze the Oris™ Universal Cell Migration Assembly Kit, it is important to stain cells using a fluorescence reagent that uniformly stains cells. The use of a fluorescence probe that is affected by experimental conditions will increase variability of results and reduce correlation between fluorescence signal and cell migration. Fluorescence probes that are affected by experimental conditions could be used, however, as counterstains for the study of factors and processes affecting cell migration.

You have successfully determined the optimal cell seeding concentration for your cell line. Proceed to Step 6 of the Oris™ Universal Cell Migration Assembly Kit Protocol.