Oris™ 3D Embedded Invasion Assay

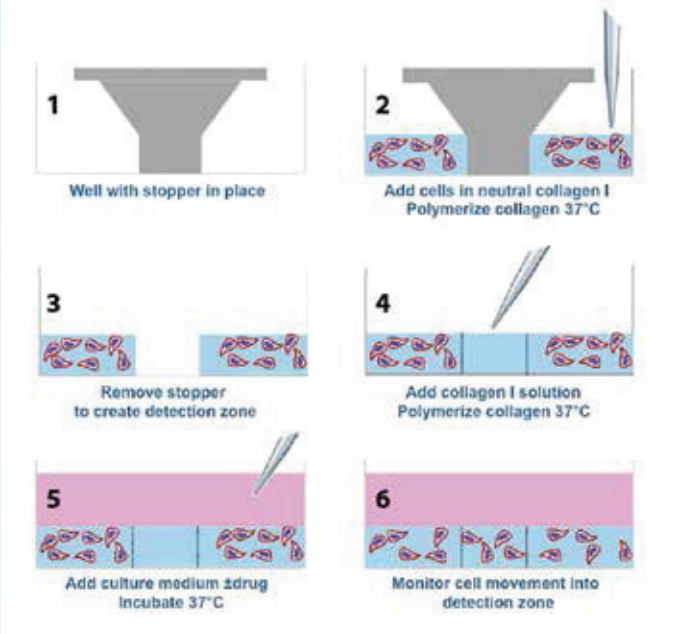
Monitor Cell Movement Through Collagen I Entirely in 3D

Oris™ Product Overview

Oris™ assays employ exclusion zone technology to facilitate unambiguous monitoring of cell migration from the periphery into a central circular detection zone. No cell tracking systems are needed to determine whether cells moved.

3D Invasion Assay Overview

The Oris™ 3D Embedded Invasion Assay plates are provided with a Collagen I coating and, except for the starter kit, with stoppers positioned in all 96 wells, ready for cell seeding:



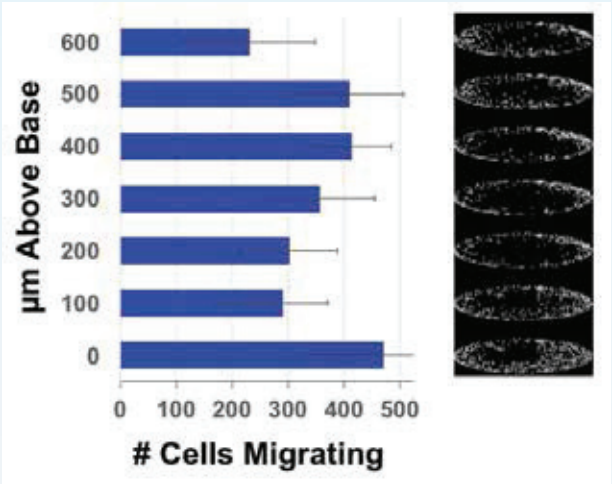
Movement of cells through the collagen can be assessed microscopically or using plate readers. For microscopy, counts of cells in multiple focal planes are the preferred approach to quantification; these counts may be summed for each well or plotted individually. For those without suitable microscopes, cells may be stained and cell movement quantified using a plate reader in conjunction with the supplied Oris™ Mask.

3D Cell Distribution

To evaluate vertical distribution of cells in the assay, 30,000 HT1080 cells in 40 µL neutralized collagen I solution were seeded into each well of an Oris™ 3D Embedded Invasion Assay plate. Embedded cells were incubated at 37°C/5% CO_2 for 1 hour to polymerize the collagen.

Stoppers were removed and 10 µL of fresh neutralized collagen I was pipetted into the empty central column and allowed to polymerize at 37°C/5% CO_2 for 1 hour to create the detection zone matrix.

Culture medium (100 µL per well) was then added and plates were incubated at 37°C/5% CO_2 for 5 days:



3D Cell Distribution: Cells were fixed with glutaraldehyde and stained with DAPI, then imaged with a Zeiss Axio Observer Z.1 inverted microscope with automated stage, capturing images of each well at 0-600 µm above the plate bottom in 100 µm increments. Cell counts were determined using ImageJ (free download from imagej.nih.gov/ij) to define the detection zone and to count in-focus cells in each plane using a brightness threshold, averaging four wells per data point. The images to the right of the histogram show ImageJ reconstructions of in-focus cells at each height.

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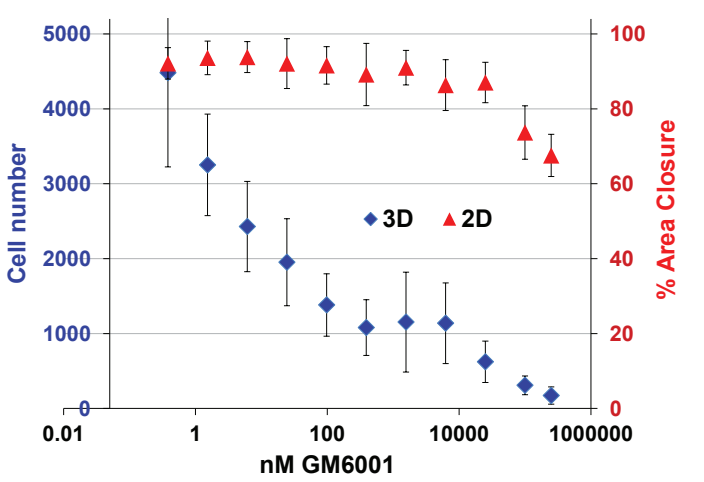
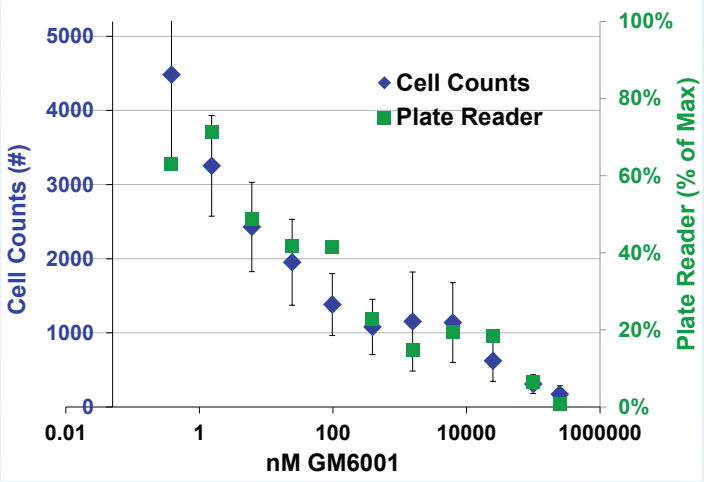
Cell Movement in the Exclusion Zone is Specifically Inhibited by MMP Inhibitor GM6001

Matrix metalloproteinase (MMP) inhibitor GM6001 blocks 3D movement of HT1080 cells through the collagen matrix in the Oris™ 3D Embedded Invasion Assay but not cell movement in the Oris™ Cell Migration Assay that monitors 2D migration across the plate surface:



Quantification Using Plate Readers Yields Comparable Results to Cell Count Data

The Oris™ 3D Embedded Invasion Assay plate from the GM6001 experiment at left was stained with DAPI and the Oris™ Mask was affixed to the plate bottom. Fluorescence signal was quantified on a BioTek Synergy® HT plate reader:

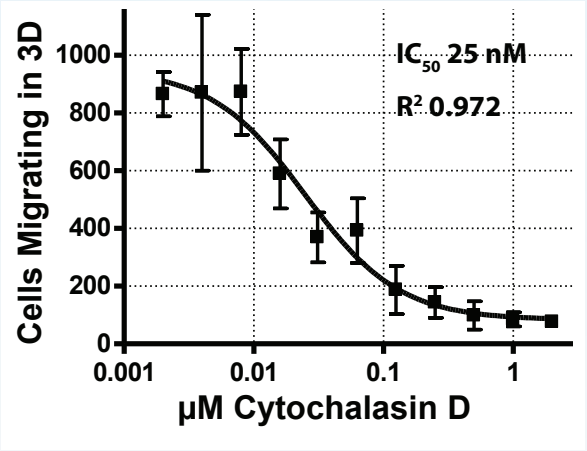


30,000 HT1080 cells were seeded per well in Oris™ 3D Embedded Invasion Assay plates (3D, blue) or Oris™ Cell Migration Assay (2D, red) plates. Culture medium +/- drug was added to wells after collagen in the central detection zone was allowed to polymerize (3D) or after stoppers were removed following cell adhesion (2D). After 1 day (2D) or 7 days (3D) cells were fixed with glutaraldehyde and stained with DAPI. All wells were imaged on a Zeiss Axio Observer Z.1 microscope with automated stage, and images were analyzed using ImageJ. For the 3D assay, invasion was quantified either (i) by counting cells in each of 11 focal planes at 0-1000 µm above the plate bottom in 100 µm increments, summing cells in each plane for each well, or (ii) using the mask and plate reader to measure fluorescence intensity in the exclusion zone. For the 2D assay, migration was quantified by measuring percent area closure in the exclusion zone. All data points are average of six wells ± standard deviation.

IC_{50} Measurements

As with all Oris™ assays, the 3D Embedded Invasion Assay can be used for measurement of the 50% inhibitory concentration (IC_{50}) of drugs that inhibit cell movement.

Measurement of IC_{50} for cytochalasin D. For the experiment charted at right, 30,000 HT1080 cells were seeded per well in the Oris™ 3D Embedded Invasion Assay. Cytochalasin D was added with culture medium after the collagen in the central exclusion zone had polymerized. Each data point represents four replicate wells ± standard deviation. Note that IC_{50} values for invasion through a collagen matrix are likely to differ from IC_{50} values even for the same drug and cell type obtained for 2D migration assays. An extreme example of this difference is the GM6001 result above.



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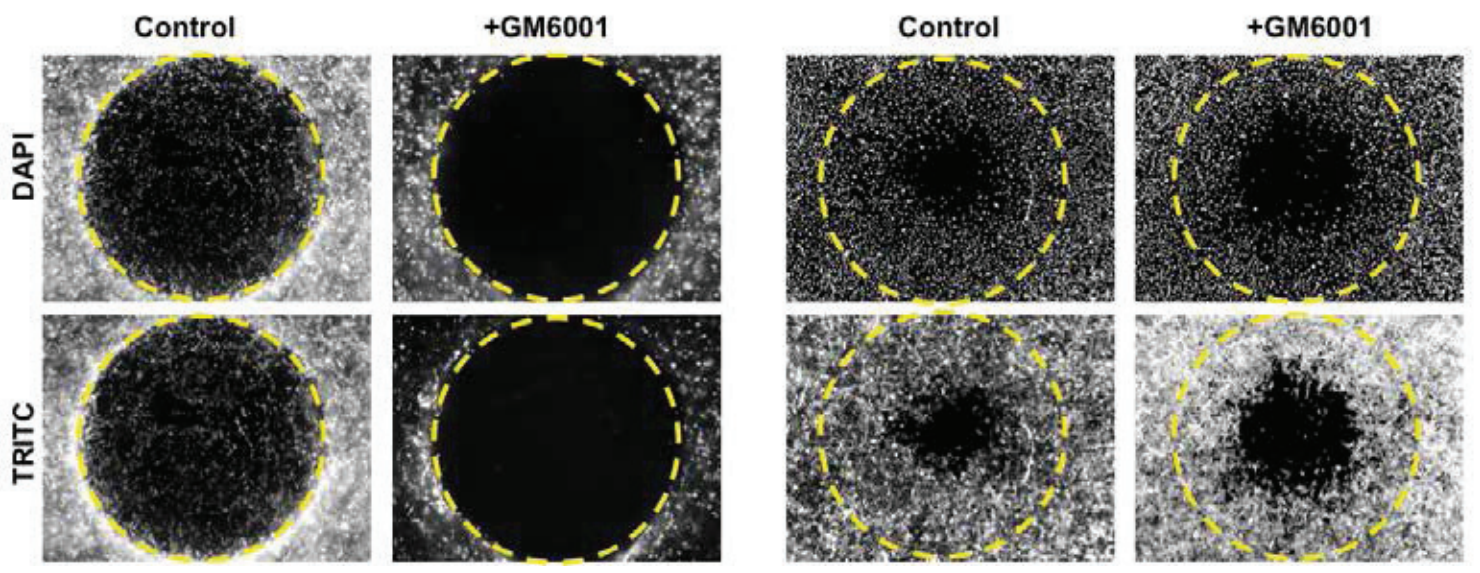
Images of cells in the 2 mm detection zone

Quantifying assay results by counting cells in the exclusion zone (yellow dotted circles) is best done with a nuclear stain such as DAPI (top row of images), whereas quantification by fluorescent plate readers is best done by staining a broader area of the cells such as the actin cytoskeleton, here stained with TRITC-Phalloidin (bottom row), which yields a stronger fluorescence signal:



3D invasion assay after 7 d incubation. GM6001 at 250 µM completely inhibited HT1080 cell invasion.

2D migration assay after 1 d incubation. GM6001 at 250 µM did not inhibit HT1080 cell migration.

FAQs

Can I use a different collagen supply or other matrix instead of the collagen provided with the assay kits?

You may use your own matrix material, however Platypus cannot guarantee a successful outcome. The collagen provided with the product is thoroughly tested in a 3D invasion assay using the recommended protocol provided. Any changes to the protocol or the matrix material will measurably affect the results.

How long does the assay take?

For best results, several days of incubation will be needed even for the most aggressively invasive cells. Although cell movement into the exclusion zone is detectable within 1-2 days, it can take several days longer for cell numbers to increase sufficiently for statistically significant numbers of cells to invade. You may need to change culture medium multiple times before the assay is finished. However, since the assay can be assessed in real time, you can view the cells as often as you like without disturbing assay progress.

Can the assay be used for co-cultures?

This is feasible if you have a means to distinguish the two cell types, such as unambiguous inherent morphological differences or cell-specific stains. If you wish to inoculate one cell type in the outer ring and another cell type in the center, note that the outer ring has a 40 µL volume whereas the inner ring is only 10 µL – cell numbers should be adjusted accordingly.

How come you don't offer invasion assays with 24 wells?

Larger wells require more cells and greater quantities of expensive reagents, so the 96-well format saves you money. Moreover, the assay will take longer in 24 well plates as invasion distances are greater, so the Platypus assay saves time too.

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ORDERING INFORMATION

Oris™ 3D Embedded Invasion Assay, 1-Pack

One 96-well plate with stoppers in position in each well,
2x 2-mL tubes of 5 mg/mL Collagen I (rat tail), qualified for invasion

Order catalog number EIA1

Oris™ 3D Embedded Invasion Assay, 3-Pack

Three 96-well plates with stoppers in position in each well,
6x 2-mL tubes of 5 mg/mL Collagen I (rat tail), qualified for invasion

Order catalog number EIA3

All three kits include

- An Oris™ Stopper Tool to facilitate removal of stoppers without disturbing the collagen gel
- A 96-well Oris™ Mask to facilitate quantification of fluorescence in the exclusion zone using plate readers
- The recommended protocol with stepwise directions for completing the assay
- Platypus expert technical support

How To Order

Via the web site, with credit card:
From www.assayhub.com

Via phone: Call 1.866.296.4455 or 1.608.237.1270

Via fax: 1.608.237.1271

Via email: orders@platypustech.com

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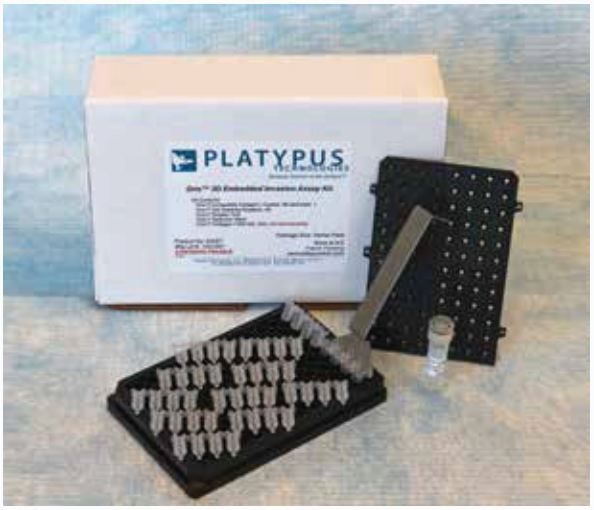
Oris™ 3D Embedded Invasion Assay Starter Kit

One 96-well plate with 48 stoppers aseptically packaged,
1x 2-mL tube of 5 mg/mL Collagen I (rat tail) , qualified for invasion

Order catalog number EIAST

The starter kit is provided for new users who wish to test-drive the assay at lower cost, using 48 of the 96 available wells.



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