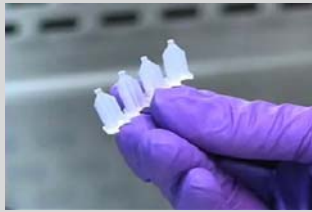
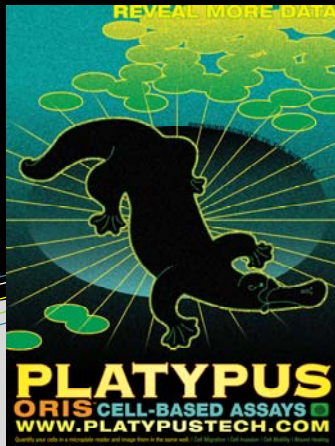
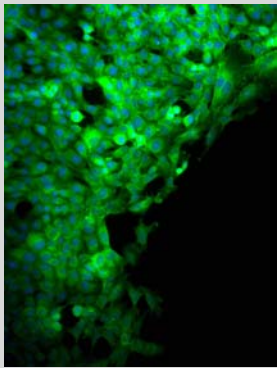


Oris™ Cell Invasion & Detection Assay



ORIS™ CELL SEEDING STOPPERS (patent pending)



MULTIPLEXED CELL STAINING



ORIS™ CELL INVASION & DETECTION ASSAY

Image & Quantify All in the Same Well

Cell Invasion is measured *in vitro* by the ability of adherent cells to move through a 3-D extracellular matrix (ECM) that mimics an *in vivo* environment^{1,2}. The Oris™ Cell Invasion & Detection Assay offers a versatile method for imaging and measuring cells invading through a 3-D ECM in real-time. The Oris™ Assay design uses patent-pending, cell seeding stoppers to create a 2 mm diameter detection zone at the center of a microplate well. Cells can be labeled with Calcein AM and those that have invaded into the detection zone can be imaged by use of a microscope or a digital imaging system. Results can then be confirmed quantitatively by using a fluorescence microplate reader.

Unlike transmembrane inserts, the Oris™ Invasion & Detection Assay is compatible to high throughput and high content screening applications. The Oris™ Assay design allows researchers to observe and quantify cell invasion in a more native, 3-D environment since cells do not invade through a porous membrane. In addition, the Oris™ Invasion & Detection Assay permits further study of specific mechanisms of cell invasion through staining of proteins associated with proteolysis and cytoskeletal structures in the same well. The unique Oris™ Assay design now makes it possible to reveal more data per well by use of microplate reader analysis and cytochemical staining.

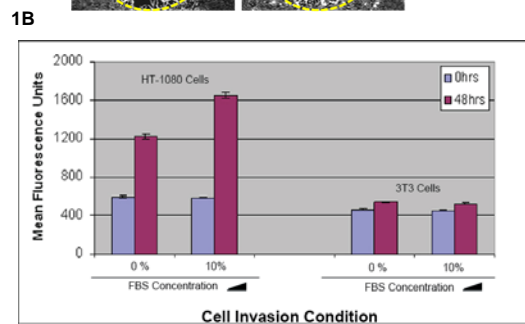
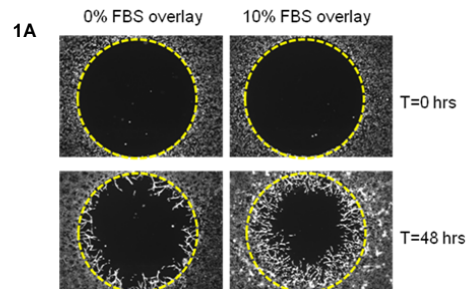


Figure 1A & 1B. Microscope & Microplate Reader Data from HT-1080 Cell Invasion. HT-1080 cells, serum starved for 18 hrs, were seeded (50,000 cells/well) on the Oris™ BME (Basement Membrane Extract) coated microplate and allowed to adhere overnight. Stoppers were removed and cells were overlaid with BME in the presence and absence of 10% FBS. After a 48-hour incubation, cells were stained with the Calcein AM reagent and imaged by microscopy (1A). The detection mask was applied to the bottom of the microplate and fluorescence from the cells in the detection zone was quantified by using a microplate reader (1B). Each column represents the mean +/- SEM of at least 22 wells. A non-invasive cell line, 3T3-Swiss albino fibroblasts, served as a negative control.

Product Highlights

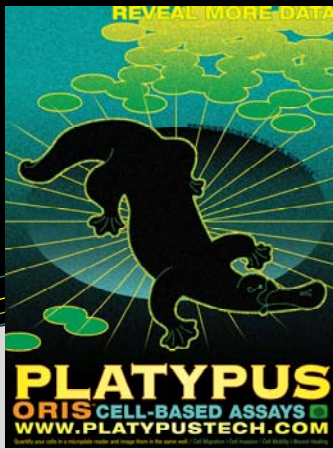
- **Generate More Data per Well** - Analyze cells treated with multiple fluorescent probes, labels or stains by using a microplate reader, microscope or high content imaging system.
- **Membrane-free Cell Invasion** - No cumbersome cell culture inserts or transmembrane devices to hinder data collection; compatible with HTS and HCS applications.
- **Preserve & Observe Cell Morphology** - A more native 3-D environment where cells do not invade through a porous membrane.
- **Real-time Monitoring** - No membrane to prohibit visualization or quantitation of cell activity; permits kinetic and endpoint assays.

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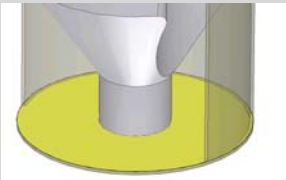
PLATYPUS TECHNOLOGIES

Product Details & Data

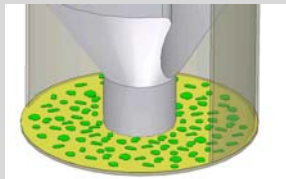


Oris™ Assay Steps

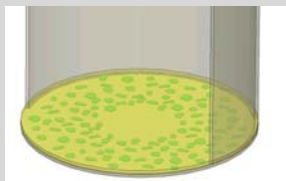
Apply BME Coating & Populate Plates with Oris™ Cell Seeding Stoppers



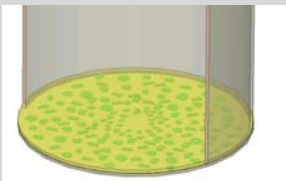
Seed & Adhere Cells onto Oris™ Plate



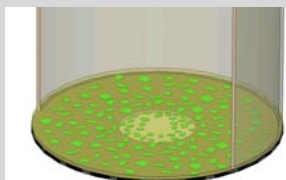
Remove Stoppers to Create Detection Zone & Apply BME Overlay



Incubate and Allow Cells to Invade into Detection Zone



Analyze Detection Zone



Characterization of HT-1080 Invadopodia

Invadopodia are cell membrane protrusions associated with proteins involved in proteolysis of the ECM and play a major role in the reorganization of the tumor microenvironment. As shown below, the Oris™ Cell Invasion & Detection Assay was used to demonstrate the co-localization of F-actin (a cytoskeletal protein necessary for cell motility) and MMP-9 (a protease involved in matrix degradation in cancer cell invasion) that is characteristic of invadopodia structures^{3,4}.

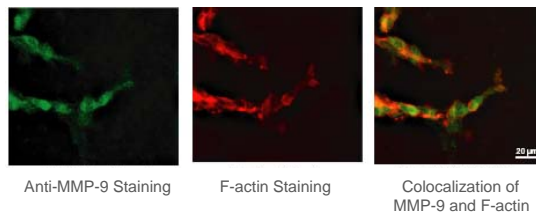


Figure 2. Cytochemical Staining of HT-1080 Cells. HT-1080 cells, serum starved for 18 hours, were seeded (50,000 cells/well) on an Oris™ BME coated plate and allowed to adhere overnight. Stoppers were removed and the cells were overlaid with 40 µl of BME. After a 48-hour incubation, the cells were stained with polyclonal anti-MMP-9 tagged with Alexa Fluor® 488 2° antibody, and Alexa Fluor® 555 phalloidin, Invitrogen.

Product Listing

PROD. NO.	DESCRIPTION	SIZE
CIA101DE	Oris™ Cell Invasion & Detection Assay Oris™ compatible 96-well plate, 1 Oris™ Cell Seeding Stoppers, 96 Oris™ Detection Mask, 1 Oris™ Stopper Removal Tool, 1 Oris™ Basement Membrane Extract (BME), 5 mL Calcein AM Reagent, 20 µl	1 plate
CIA200DE	Oris™ Cell Invasion & Detection Assay Oris™ compatible 96-well plate, 2 Oris™ Cell Seeding Stoppers, 2 x 96 Oris™ Detection Mask, 2 Oris™ Stopper Removal Tool, 2 Oris™ Basement Membrane Extract (BME), 2 x 5 mL Calcein AM Reagent, 2 x 20 µl	2 plates

Additional Oris™ Cell Based Assays Available at www.platypustech.com

Alexa Fluor® is a trademark of Invitrogen.

Inhibition Studies of Cell Invasion

The effects of inhibitors on MDA-MB-231 breast epithelial cell invasion were studied by using the Oris™ Cell Invasion & Detection Assay. Inhibitors included: Blebbistatin, a selective inhibitor of myosin II ATPase, and H-1152, a potent Rho-kinase (ROCK) inhibitor. Images below indicate that the Rho-kinase pathway is important for invasion of MDA-MB-231 cells.

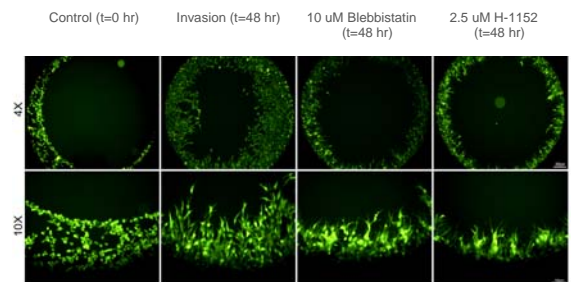


Figure 3. Inhibition of MDA-MB-231 Cell Invasion. MDA-MB-231 cells were seeded (25,000 cells/well) on an Oris™ BME coated plate and allowed to adhere overnight. Stoppers were removed, cells were overlaid with 40 µl of BME, and media supplemented with inhibitors was added. After a 48-hour incubation, the cells were labeled with Calcein AM and imaged.

References

- Liotta, L.A. 1984. *Tumor invasion and metastasis: role of the basement membrane.* Am. J. Pathol. 117:339-348.
- Terranova, V.P., E.S. Hujanen, D.M. Loeb, G.R. Martin, L. Thornburg, and V. Glushko. 1986. *Use of reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells.* Proc. Natl. Acad. Sci. USA 83:465-469.
- Weaver, AM; Clin Exp Metastasis 2006. *Invadopodia: specialized cell structures for cancer invasion.* 23:97-105.
- Furmaniak-Kazmierczak et al; Circulation Research 2007. *Formation of extracellular matrix-digesting invadopodia by primary aortic smooth muscle cells.* 100:1328-1336.

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