QUANTIFYING CELL MIGRATION IN REAL-TIME USING THE ORIS™ CELL MIGRATION ASSAY

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Application Note

Introduction

Cell migration is critical for many physiological events, including embryonic development, wound healing, and the inflammatory response. Furthermore, aberrant motile behavior of cells contributes to pathological processes including tumor metastasis and arthritis [1].

The Oris™ Cell Migration Assay (Figure 1) uses a 96-well plate populated with silicone stoppers that exclude cells from a central region in each well. After cells are seeded and allowed to adhere to the well, the stoppers are removed to create a 2 mm diameter Detection Zone into which cells are permitted to migrate.
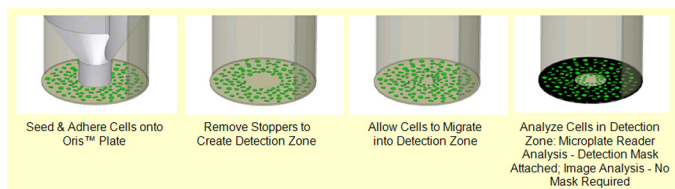

Seed & Adhere Cells onto Oris™ Plate | Remove Stoppers to Create Detection Zone | Allow Cells to Migrate into Detection Zone | Analyze Cells in Detection Zone: Microplate Reader Analysis - Detection Mask Attached; Image Analysis - No Mask Required

Figure 1. Oris™ Cell Migration Assay Schematic

This application note describes a method to quantify the number of cells that have migrated into the Detection Zone using a fluorescence microplate reader. By first creating a standard curve of fluorescence intensity to number of stained cells per well, a second curve can then be generated to determine the number of stained cells that have migrated into the Detection Zone at any given time point.

Materials & Methods

In this application, 3T3-Swiss albino fibroblast cells were fluorescently labeled with CellTracker™ Green (Invitrogen), a live cell stain, and then seeded and allowed to migrate on the Oris™ Cell Migration Assay plate. In parallel, a standard curve was generated to determine the actual number of cells that migrated into the Detection Zone based on fluorescence intensity. After 21 hours, cell migration into the Detection Zone was measured by obtaining Relative Fluorescence Unit (RFU) values from a Synergy™ HT Multi-Mode Microplate Reader (BioTek). These RFU values were used to establish the standard curve and generate standard regression equations to determine the number of migrating cells.

Preparation of Cells and Suggested Plate Layout:

1. Expand the cell line of interest to 70 – 80% confluency.

2. Add an appropriate amount of CellTracker™ Green (5 ng/mL) to the cells. Place the cells with CellTracker™ Green into an incubator for 30 minutes.

3. Upon removal from the incubator, remove medium containing CellTracker™ Green and replace with cell culture medium. Return cells to the incubator for 30 minutes.

4. Remove cells from the incubator, detach the cells, and count using a hemocytometer.

5. A suggested Oris™ plate layout is shown in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution 1	Dilution 1	Dilution 1	Dilution 1	Test	Test						
B	Dilution 2	Dilution 2	Dilution 2	Dilution 2	Test	Test						
C	Dilution 3	Dilution 3	Dilution 3	Dilution 3	Test	Test						
D	Dilution 4	Dilution 4	Dilution 4	Dilution 4	Test	Test						
E	Dilution 5	Dilution 5	Dilution 5	Dilution 5	Test	Test						
F	Dilution 6	Dilution 6	Dilution 6	Dilution 6	Test	Test						
G	Dilution 7	Dilution 7	Dilution 7	Dilution 7	Test	Test						
H	Media Only	Media Only	Media Only	Media Only	Test	Test						

Table 1. Suggested Oris™ Plate Layout

Preparation of Cell Suspensions & Cell Attachment:

6. Using the optimum cell seeding concentration previously determined for the cell line of interest, prepare a >1.6 mL cell suspension. Add 100 uL of this cell suspension to each well in Columns 5 and 6.

Note: These wells will serve as the test wells for quantitating the number of cells in the Detection Zone of the cell migration assay (cell migration curve).

7. Remove Oris™ Cell Seeding Stoppers from the wells of Columns 1-4. Starting with the same cell concentration as that used in step 6, prepare >0.4 mL each of 7 two-fold serial dilutions and a media-only blank (see Table 1). Add 100 uL of the serial dilutions and the media blank into the wells of Columns 1-4.

Note: These wells will be used to prepare the standard curve.

8. Place the Oris™ plate into an incubator for 2-4 hours to allow cell attachment. Incubation time(s) will be cell line dependent.

9. Once the cells have attached, remove the Oris™ Cell Seeding Stoppers from the wells of Columns 5 and 6.

10. Remove medium from all wells and replace with phenol red-free medium. Cell proliferation should be arrested in standard curve wells (Columns 1-4) through the use of an

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Data Collection:

11. Attach the Oris™ Detection Mask to the bottom of the Oris™ plate. Using optimized microplate reader settings, obtain the RFU values for each well. Readings obtained immediately after removal of the Oris™ Cell Seeding Stoppers will be the t = 0 values. Continue acquiring RFU values at defined intervals, such as t=1, 2, 4 and 24 hours. Make sure to return the Oris™ plate back to the incubator in between readings.

Data Analysis:

12. Using the RFU data obtained from Columns 1 - 4, tabulate the Number of Cells Seeded/Well and the Average RFU/Well values for each time point (Table 2).

Note: Each aperture of the Detection Mask exposes 9.5% of the total well bottom to the microplate reader. Therefore, to calculate the Theoretical Number of Cells in the Detection Zone, multiply the Number of Cells Seeded by 0.095; record these values (refer to Table 2).

The Average RFU/Well values correspond to the Number of Cells in the Detection Zone. Compile a new data table for each time point.

	Number of Cells Seeded/Well	Theoretical Number of Cells in the Detection Zone (Number of Cells Seeded/Well x 0.095)	Average RFU/Well at t=21 hrs (n=4)
Dilution 1	25,000	2,375	1,820
Dilution 2	12,500	1,188	901
Dilution 3	6,250	594	527
Dilution 4	3,125	297	333
Dilution 5	1,563	148	260
Dilution 6	781	74	212
Dilution 7	391	37	197
Media Only	0	0	184

Table 2. Example Data Table Used to Generate Standard Curve at t=21 hrs

Generation of a Standard Curve:

13. Generate a standard curve by plotting the Number of Cells in Detection Zone (x-axis) versus Average RFU units (y-axis), as shown in Figure 2.
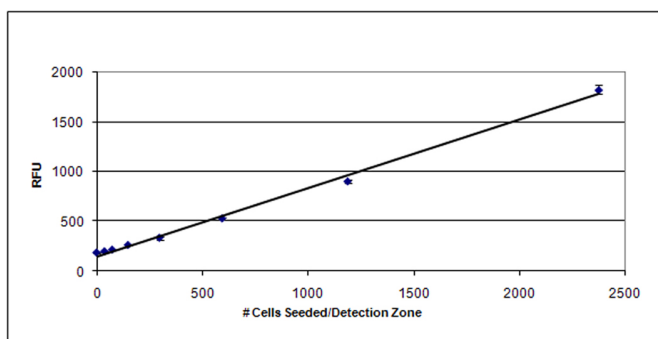

Figure 2. Sample Standard Curve at t=21 hours.

14. From the standard curve, calculate a regression equation for each timepoint. For the dataset used in Figure 2, the regression equation is y=0.686x + 149.976.

15. Generate a separate standard curve and regression equation for each time point at which RFU values were collected.

Creation of Migration Curve:

16. Using the data obtained from the test wells (Columns 5 and 6, n=16), calculate an average RFU value for each time point. Once the average RFU values have been determined, use the calculated regression equation for each time point (calculated from the standard curve at that same time point) to determine the number of cells present in the Detection Zone at each separate time point, as shown in Table 3.

Time Point (hours)	Linear Regression Equation	Average RFU / Well n=16	Calculated Number of Cells in Detection Zone
0	y = 0.689x + 151.994	299	215
1	y = 1.366x + 141.063	375	347
2	y = 0.662x + 133.780	426	441
3	y = 0.665x + 133.560	449	475
4	y = 0.673x + 134.381	484	519
6	y = 0.665x + 133.991	540	519
21	y = 0.686x + 149.976	795	939

Table 3. Tabulation of Number of Cells in Detection Zone at various time points.

17. Prepare a graph illustrating cell migration over time by plotting The Calculated Number of Cells in Detection Zone (y-axis) versus Hours (x-axis), as shown in Figure 3.
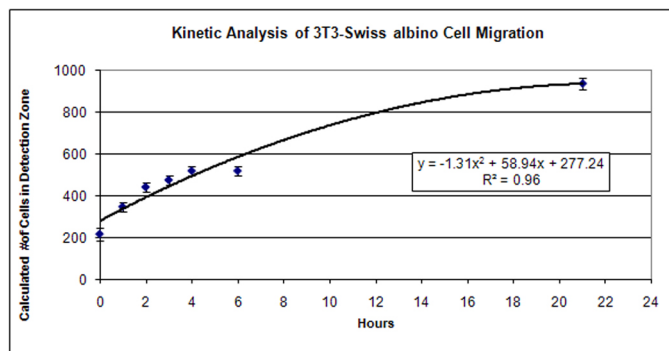

Figure 3. Quantitation of 3T3-Swiss albino Cell Migration over Time

Conclusions

This application note describes a method to quantify in real-time the number of cells that have migrated into the Detection Zone using a fluorescence microplate reader. Using this method, one can extrapolate the number of cells that have migrated at any time point during the assay.

CellTracker™ Green is a trademark of Invitrogen Corporation.

Synergy™ HT is a trademark of BioTek Instruments, Inc.

[1] Ridley et al., 2003. Science. 302(5651):1704-1709.

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