Product Manual

Cell Migration BioGel Assay, Tissue Culture Treated

Catalog #: ENZ-KIT109

ENZ-KIT109-0001 1x96 wells

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

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

TABLE OF CONTENTS

INTRODUCTION

The Cell Migration BioGel Assay is a reproducible, sensitive, and flexible assay that can be used to monitor cell migration. Formatted for a 96-well plate, the assay uses a non-toxic biocompatible gel (BioGel) to form a cell-free zone on cell culture surfaces. After seeding cells into the 96-well plate, the BioGel dissolves permitting cells to migrate into the well centers (see Figure 1). The Cell Migration BioGel Assay enables the use of automated liquid handling equipment for cell seeding and allows for unlimited access to wells from cell seeding through data readout. The Cell Migration BioGel Assay is designed to be used with any commercially available stain or labeling technique. Researchers can capture and quantify real-time cell migration data using inverted microscopes, High Content Screening (HCS) and High Content Imaging (HCI) instruments.

The Cell Migration BioGel Assay system has been designed for use with adherent cell cultures. This assay has been successfully used with HT-1080 and MDA-MB-231 cell lines, and Human Umbilical Vein Endothelial Cells (HUVECs).

Using the Cell Migration BioGel Assay offers the following features & benefits:

- **Increased Productivity** - Treat cells with multiple fluorescent probes, labels, or colorimetric stains for multi-parametric measurements with inverted microscopes, High Content Screening (HCS) and High Content Imaging (HCI) instruments.
- **Less Handling** - Realize reduced assay handling time with an assay format in which a centrally placed biocompatible gel automatically dissolves to reveal a detection zone.
- **Automation-Friendly Design** - Utilize automated liquid handling equipment for fast set-up of high throughput, 96-well assays.
- **Reproducible Results** - Achieve well-to-well CV's ≤12% and generate robust Z’ factors suitable for compound screening.
- **Real-Time Analysis** - Experience unlimited access to cells, cell morphology and cell movement throughout your experiment.

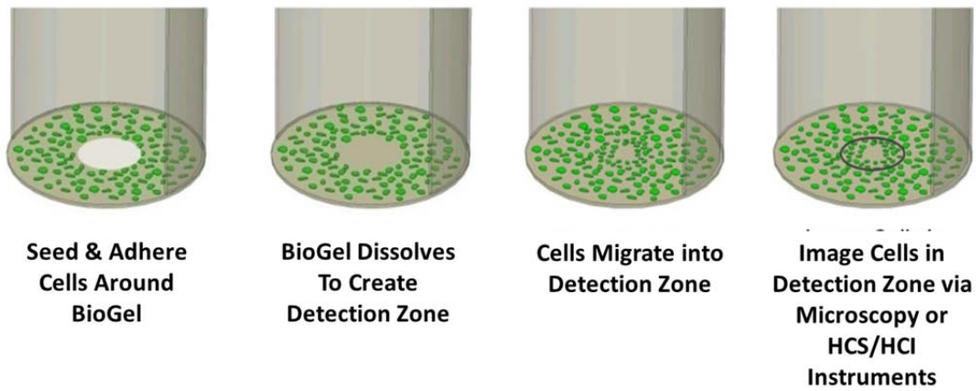

Figure 1. Schematic of Cell Migration BioGel Assay

CELL MIGRATION BIOGEL ASSAY PLATE DIMENSIONS

Diameter of Well - Bottom	6.58mm
Diameter of Well - Top	6.96mm
Well Volume	392µl
Suggested Media Volume per Well	100µl
Plate Height	14.4mm
Plate Height with Lid	17mm
Offset of Wells (A-1 location, X)	14.38mm
Offset of Wells (A-1 location, Y)	11.24mm
Distance between Wells	9.0mm
Well Depth	10.9mm
Thickness of Well Bottom	190µm +/- 10µm
Storage Conditions	15–30°C

NOTE: For Research Use Only.

Important: Read instructions before performing any assay.

MATERIALS PROVIDED

(1) Cell Migration BioGel Assay, Tissue Culture Treated 96-well Plate

MATERIALS REQUIRED

- Biological Cells
- Sterile PBS (containing both Ca^{++} and Mg^{++})
- Complete Cell Culture Growth Medium (containing serum)
- Pipette or Multi-Channel Pipette with Sterile Pipette Tips
- Trypsin or Cell Scraper
- Inverted Microscope (optional)
- High Content Screening, High Content Imaging System (optional)
- Cell Culture Labeling Medium, e.g., phenol red-free/serum-free media (optional)
- Cell Labeling Fluorescent Agent (eg., Cyto-ID® Green Cell Tracer Kit, ENZ-51036 or Cyto-ID® Red Cell Tracer Kit, ENZ-51037), DAPI, TRITC-Phalloidin (optional)(required if performing assay readout via fluorescence analysis)

CELL MIGRATION BIOGEL ASSAY PROTOCOL

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

1. If performing a kinetic analysis of cell migration, pre-label cells with a fluorescent stain now. Please refer to **Appendix II** for a discussion of suggested staining techniques.

2. Collect cells and prepare a suspension that is at 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 refer to **Appendix I** for a discussion of this process.

3. Pipette 100µl of suspended cells into each test well.

NOTE: If you plan to fix and label test cells at the conclusion of the cell migration, you will need additional wells (or an additional Cell Migration BioGel Assay plate) to serve as pre-migration reference wells.

NOTE: Place your seeded plate(s) into the incubator as soon as possible after cells have been seeded. Take care not to jostle the plate(s).

4. Incubate the seeded plate(s) containing the Cell Migration BioGel (see **Figure 2**) in a humidified chamber (37°C, 5% CO_2) for 1 to 4 hours (cell line dependent) to permit cell attachment.

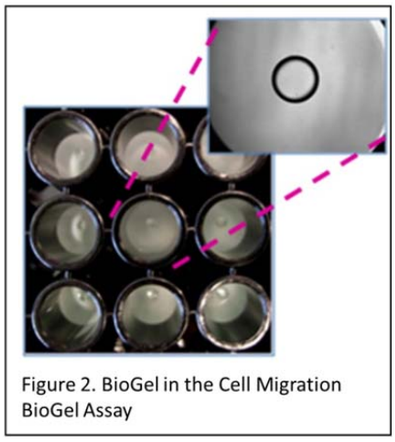

Figure 2. BioGel in the Cell Migration BioGel Assay

5. Remove plate(s) from incubator.

NOTE: At this step, test compounds may be added directly to the well, or it may be preferable to first remove media and add fresh culture media containing test compounds to each well.

6. Capture pre-migration images of the Detection Zone (to be used as reference wells) according to the following options:

Option I: If utilizing unlabeled cells or live, labeled cells (GFP-labeled, or a non-toxic fluorescent dye, such as Cyto-ID®), use an inverted microscope or HCS/HCI instrument to capture pre-migration images of the Detection Zone formed in the wells.

Option II: If utilizing fixed, labeled cells (TRITC-phalloidin, DAPI, etc), fix cells in the pre-migration reference wells. These cells can be labelled immediately or at the same time as the test cells. Use an inverted microscope or HCS/HCI instrument to capture pre-migration images of the Detection Zone formed in the wells.

7. Incubate plate in a humidified chamber (37°C, 5% CO_2) to permit cell migration. Cells may be examined by inverted microscope or other imaging instrument throughout the incubation period to monitor progression of migration, which will vary depending upon cell type and experimental design.

8. If performing an endpoint analysis of cell migration, stain cells with a fluorescent stain after sufficient migration has occurred. Refer to **Appendix II** for further information on fluorescence staining techniques of fixed cells.

9. Capture post-migration images of the Detection Zone using HCS/HCI instrumentation, or phase, bright-field, or fluorescence microscopy.

DATA ACQUISITION

The readout of the Cell Migration BioGel Assay can be conducted at any time, thereby allowing the user to perform a kinetic assay or an endpoint assay. The Cell Migration BioGel Assay is designed to be used with any commercially available stain or labeling technique. Readout can be performed by using an inverted microscope or a High Content Screening (HCS) or High Content Imaging (HCI) instrument.

Microscope Analysis:

- Cell counting or image capture/analysis software, such as NIH ImageJ freeware, can be used.
- **Note**: Microscopy observations are possible using phase contrast, fluorescence or bright field microscopy.

Sample data using phase contrast microscopy (Figure 3). Wells were seeded with 30,000 HT-1080 cells (i.e., 100µl of $3.0x10^5$ cells/ml) and incubated (37°C, 5% CO_2) for 2 hours. Upon removal of the plate from the incubator, phase contrast images were taken for pre-migration references. The plate was returned to the incubator for 24 hours to permit cell migration. At the end of the incubation, images of cell migration were captured using phase contrast microscopy. The images below illustrate representative data from pre-migration (t=0 hrs) and post-migration (t=24 hrs) wells (CV of 7% Δ migration and Z' = 0.5 for migration*). Scale bar = 500µm.

** Reference:Zhang JH, Chung TD, Oldenburg KR, "A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays." J Biomol Screen. 1999; 4(2):67-73.*

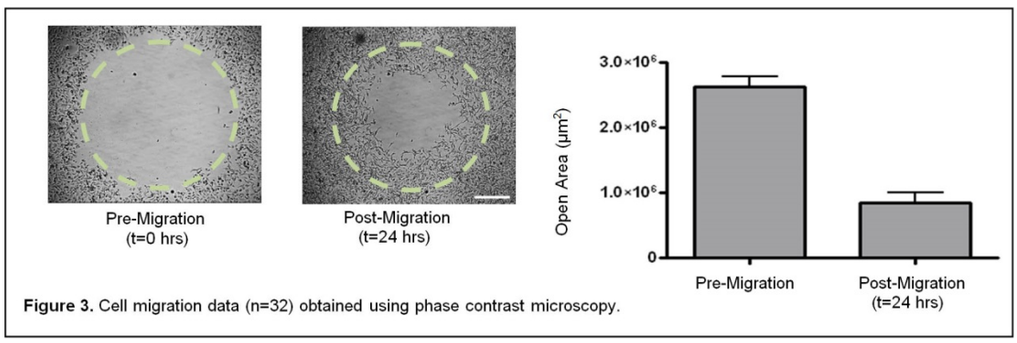

Figure 3. Cell migration data (n=32) obtained using phase contrast microscopy.

Sample data using fluorescence microscopy (Figure 4). Wells were seeded with 25,000 HUVECs (i.e., 100µl of $2.5x10^5$ cells/ml) on two plates and incubated (37°C, 5% CO_2) for 2 hours. At the end of the incubation, one plate was removed from the incubator, fixed, and stained for F-actin (TRITC-phalloidin, pseudocolored green). The second plate was incubated for an additional 24 hours to permit cell migration. At the end of the migration, the second plate was fixed and stained for F-actin (TRITC-phalloidin, pseudocolored green). Images of cell migration were captured using fluorescence microscopy and the images below illustrate representative data from pre-migration (t=0 hrs) and post-migration (t=24 hrs) wells (CV of 5.7% Δ migration and Z' = 0.77 for migration). Scale bar = 500µm.

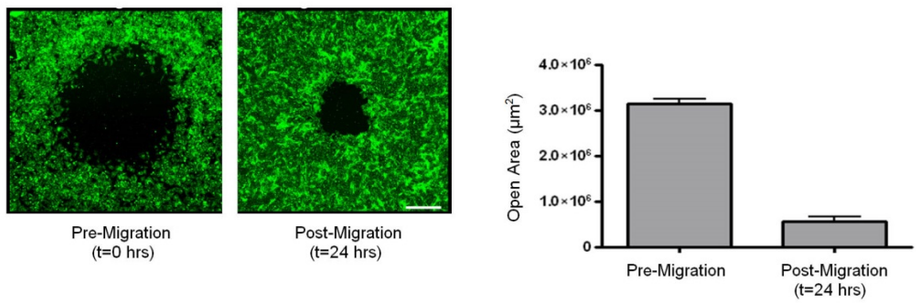

Figure 4. Cell migration data (n=32) obtained using fluorescence microscopy.

High Content Screening / High Content Imaging Analysis:

Sample data using BD Pathway™ 855 Bioimaging system (Figure 5). Wells were seeded with 25,000 HUVEC's (i.e., 100µl of $2.5x10^5$ cells/ml) on two plates and incubated (37°C, 5% CO_2) for 2 hours. At the end of the 2 hour incubation, one plate was removed from the incubator and cells were fixed with 0.25% glutaraldehyde. At this time, varying concentrations of the MEK inhibitor, UO126, were added to select wells of the second plate and incubated for an additional 24 hours to permit cell migration. At the end of the migration, the second plate was fixed with 0.25% glutaraldehyde. Both plates were stained using TRITC-phalloidin. The images below illustrate representative data from pre-migration (t=0 hrs) and post-migration (t=18 hrs) wells. Scale bar = 500µm.

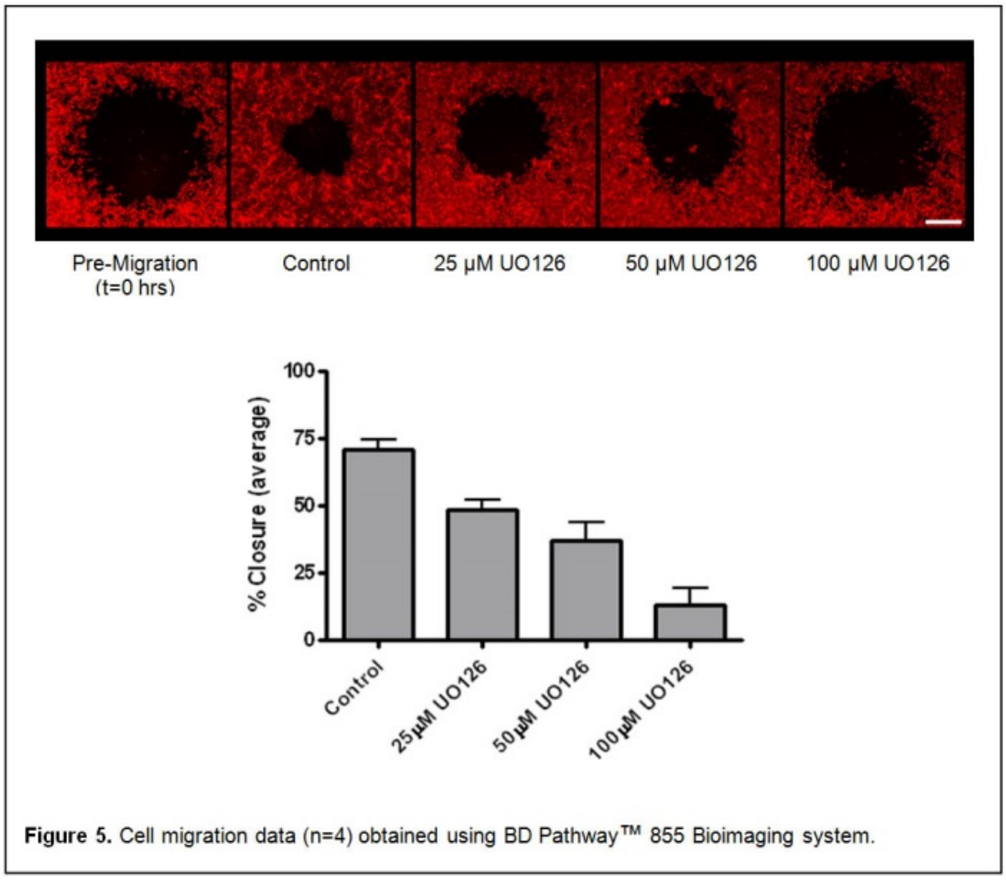

Figure 5. Cell migration data (n=4) obtained using BD Pathway™ 855 Bioimaging system.

APPENDIX I: DETERMINING OPTIMAL CELL SEEDING CONCENTRATION

This procedure is intended to assist in determining the cell seeding density needed to achieve confluency of your cell line when using the Cell Migration BioGel Assay. The intended goal is to achieve 90-95% confluency of the monolayer surrounding the BioGel without overgrowth.

1. A suggested starting point is to evaluate a range of three cell densities as shown below. The cell seeding area of the well with the BioGel is ~0.3cm^2. Based on the typical seeding density of your particular cell line, you can infer a different cell number for your first serial dilution and adjust the numbers below accordingly.
2. Prepare a log-phase culture of the cell line to be tested. Collect cells and determine the total number of cells present.
3. Pellet cells by centrifugation (1,000 x g). Prepare final concentrations of 4.0x10^5, 3.0x10^5, and 2.0x10^5 cells/ml.
4. Dispense 100µl of cell suspension per well into the 96-well plate to result in the following plate layout:

Column	1	2	3
Cells / well	40,000	30,000	20,000
Number of wells	8	8	8

5. Incubate the plate in a humidified chamber (37°C, 5% CO_2) for 1-4 hours (cell line dependent) to allow the cells to firmly attach and spread on the well surface.
6. Following cell attachment, use an inverted microscope to visually inspect each well to determine the minimum cell seeding concentration that yields a confluent monolayer at the perimeter of the Detection Zone (See **Figures 3** and **4** for representative pre-migration Detection Zone images).

At this point, you have successfully determined the optimal cell seeding concentration to be used in Step **3** of the Cell Migration BioGel Assay Protocol.

APPENDIX II: FIXATION AND FLUORESCENT LABELING OF CELLS

The Cell Migration BioGel Assay has been designed to work with all types of fluorescent stains and staining techniques. The precise method for staining cells with fluorescent stains varies according to the nature of the individual stain. Please consult the manufacturer of your fluorescent stain for specific considerations.

The following is an example Fluorescent Staining Protocol to label fixed cells with TRITC-phalloidin (F-actin) and DAPI (nuclei):

1. To fix one fully-seeded 96-well plate, prepare 10ml of fixative solution (e.g., 0.25% glutaraldehyde solution in PBS prepared from 8% EM-grade glutaraldehyde solution).
2. Remove medium and rinse wells with 100µl of 1X PBS.
3. Remove PBS and add 100µl of a fixative solution (final concentration of 0.25% glutaraldehyde solution in PBS) to each well. Incubate at room temperature for 15 minutes.
4. Remove fixative solution and rinse wells with 100µl of PBS.
5. Remove PBS and replace with 100µl of a 1:50-1:100 dilution of TRITC-phalloidin (prepared from 10µM stock in methanol) in PBS containing 0.1% Triton X-100.
6. Incubate plate at room temperature for 45 minutes (protect from light).
7. Remove the TRITC-phalloidin, rinse with PBS for 5 minutes, and add 100µl of a 1:4000 dilution of DAPI (prepared from 1 mg/ml stock) in PBS.
8. Incubate plate at room temperature for ~5 minutes (protect from light). Observe plate starting at 5 minutes and if needed allow additional time for complete staining.
9. Remove DAPI stain and wash wells 2X for 5 minutes each with 200µl of PBS.
10. Replace final wash with 200µl of fresh PBS.

NOTE: This protocol outlines double-labeling of cells with a cytoskeletal and a nuclear stain. The protocol can be simplified if only one stain is used. Substitutions, additional cytostaining, or immunostaining may be performed using non-overlapping fluorophores and by utilizing the appropriate filters with your imaging equipment.

NOTES

NOTES

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