Datasheet for ABIN5067548

Radius™ 48-Well Cell Migration Assay

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Overview

Quantity:	48 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	Radius™
Sample Type:	Cell Samples
Characteristics:	The Radius™ Cell Migration Assay Kit utilizes a proprietary 48-well plate to monitor the migratory properties of cells. Each plate well contains a 0.68 mm non-toxic, biocompatible hydrogel spot (Radius™ Gel) where cells cannot attach. When adherent cells are seeded in the Radius™ Cell Migration well, they attach outside of the Radius™ Gel coated area. Once firm cell attachment is achieved, the hydrogel is quickly removed to expose a cell-free region to study cell migration/closure. This format provides a robust in vitro system to measure 2-D cell migration, screen potential inhibitors and study cytoskeleton reorganization events. Additional features of the Radius™ Cell Migration Assay: Exclusive coating method which produces consistent gel spot size - 0.68 mm diameter ± 0.014 mm (2 %) Qualitative, quantitative, real-time or endpoint analysis Radius™ Cell Migration Plate does not need to be used all at once - unused wells can be used in future experiments, up to a total of 3 migration experiment cycles Compatible with all cell stains, dyes, and labels Complete migration zone closure achievable in 15-30 hours (actual time is cell line dependent) Analyze by phase contrast or fluorescence microscopy Compatible with HCS/HCI instrumentation Adaptable to liquid handling equipment Optimized for 10X magnification (entire Radius™ Gel migration area is viewed in a 10X magnification field) Radius™ Gel removal is controlled and extremely fast (migration starts

Product Details

	almost simultaneously between wells) The Radius™ Cell Migration Assay Kit is designed for High Content Analysis applications or imaging software and is adaptable to liquid handling equipment. Each kit provides sufficient quantities to perform 48 migration tests.
Components:	1. Radius™ 48-well Cell Migration Plate : One 48-well, tissue culture treated plate with each well containing one Radius™ non-toxic, biocompatible hydrogel spot (48 gel spots total per plate) 2. Radius™ Gel Pretreatment Solution : One Sterile Bottle - 13.0 mL 3. Radius™ Wash Solution : One Sterile Bottle - 13.0 mL 4. Radius™ Gel Removal Solution, 100X : One Sterile Tube - 150 µL 5. DAPI Fluorescence Stain, 1000X : One Amber Tube - 30 µL 6. Fixation Solution : One Bottle - 20.0 mL 4 7. Cell Stain Solution : One Bottle - 12.0 mL

Target Details

Background:	Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the attractant, these protrusions can consist of large, broad lamellipodia or spike-like filopodia. In either case, these protrusions are driven by actin polymerization and can be stabilized by extracellular matrix (ECM) adhesion or cell-cell interactions (via transmembrane receptors).

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Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Proprietary biocompatible hydrogel creates a circular area across which cells may migrate following gel removal • Versatile plate format allows use with cells of any size, no need to worry about selecting cell culture inserts with the proper pore size • Allows qualitative, quantitative, endpoint or real-time analysis • Adaptable to liquid handling equipment and HCS instrumentation
Reagent Preparation:	1X Radius™ Gel Removal Solution: Just prior to use, prepare a 1X Radius™ Gel Removal Solution by diluting the provided 100X stock 1:100 in complete culture medium. 1X DAPI Fluorescence Stain: Just prior to use, prepare a 1X DAPI Fluorescence Stain by diluting the provided 1000X stock 1:1000 in PBS.
Assay Procedure:	I. Pretreatment of Radius™ Migration Plate 1. Under sterile conditions, remove the Radius™ 48-well Cell Migration Plate from its packaging.

Note: If wells of the plate have already been used for a previous experiment, the unused wells must be completely moisture-free before proceeding. Please allow the covered plate to dry at room temperature for 1 hour. Each plate can handle up to 3 migration experiment cycles.

2. Determine which wells will be assayed (it is recommended that all samples be tested in triplicate). Slowly add 250 µL of Radius™ Gel Pretreatment Solution to each well by carefully pipetting down the wall of the well. Note: The Radius™ Gel Pretreatment Solution should not be added to any wells that will not be used immediately.
3. Cover the plate and incubate at room temperature for 20 minutes.
4. Carefully aspirate the Radius™ Gel Pretreatment Solution from the wells. Do not allow wells to dry. Note: Avoid potential damage to the Radius™ Gel Spot (located in the center) by aspirating from the edge of the well. 5
5. Slowly add 250 µL of Radius™ Wash Solution to each well. Proceed to the Cell Seeding Section below. Note: Wells can remain in the Radius™ Wash Solution for up to 1 hour.

II. Cell Seeding

• Harvest and resuspend cells in culture medium at 0.1 - 0.3 x 10 cells/mL. Note: Cell seeding density is highly cell line dependant, factoring in cell size, spreading and division. Ideally, the desired monolayer confluency at the start of migration (after Radius™ Gel Removal step) should be 80-90 % . Optimization is recommended and can be done in a standard 48-well cell culture plate prior to migration assays.

• Carefully aspirate the Radius™ Wash Solution from the wells (step 5 above). Do not allow wells to dry. Note: Avoid potential damage to the Radius™ Gel Spot (located in the center) by aspirating from the edge of the well.

• Slowly add 250 µL of the cell suspension to each well by carefully pipetting down the wall of the well.

• Transfer the plate to a cell culture incubator for 4-24 hours to allow firm attachment/spreading. Take care to avoid shaking or bumping the plate. III. Radius™ Gel Removal

1. Carefully remove the Radius™ Migration Plate from cell culture incubator.
2. Aspirate the media from each well and wash 3 times with 250 µL of fresh media. Do not allow wells to dry. Note: These washes are intended to remove debris or any dead/unattached cells.
3. Prepare sufficient 1X Radius™ Gel Removal Solution for all wells by diluting the stock 1:100 in culture medium (See Preparation of Reagents Section).
4. Aspirate the media from the wells and add 250 µL of 1X Radius™ Gel Removal Solution.
5. Transfer the plate to a cell culture incubator for 30 minutes to allow complete gel removal.
6. Aspirate the 1X Radius™ Gel Removal Solution from each well and wash 3 times with 250 µL mL of fresh media. Do not allow wells to dry.
7. After the final washing is complete, add 500 µL of complete medium to each well. Agents that inhibit or stimulate cell migration may also be added directly to the wells.
8. At this point, pre-migration images may be captured with an inverted microscope, imaging

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software, or HCI/HCS instrument.

9. Transfer the plate back to the cell culture incubator/microscope stage incubator during the migration process.
10. Monitor the migration closure by endpoint or real-time analysis. For time course experiments, live cell compatible dyes or labels are required (e.g. Calcein AM, GFP, RFP). For endpoint experiments, fixed cell detection should be used (Cell Stain, DAPI, TRITC-phalloidin). 6

IV. (Optional) DAPI Fluorescence Labeling

1. Aspirate the media from the wells and add 250 µL mL of Fixation Solution to each.
2. Allow the cells to fix for 10 minutes at room temperature. Aspirate and discard the solution.
3. Carefully wash each well 3 times with 500 µL of PBS.
4. Prepare sufficient 1X DAPI Fluorescence Stain for all wells by diluting the stock 1:1000 in PBS (See Preparation of Reagents Section).
5. Add 250 µL of 1X DAPI Stain to each well to be stained.
6. Incubate 15 minutes at room temperature.
7. Carefully wash each well 3 times with 500 µL of PBS.
8. Add 500 µL PBS to each well to keep cells hydrated.
9. Examine wells under an inverted fluorescence microscope with DAPI filter (350nm/470nm)

V. (Optional) Cell Staining

1. Aspirate the media from the wells and add 200 µL of Cell Stain Solution to each.
2. Allow the cells to stain for 15 minutes at room temperature. Aspirate and discard the solution.
3. Carefully was each well 3 times with 500 µL of deionized water.
4. Discard all washes and allow wells to dry at room temperature.
5. Examine wells under an inverted light microscope. Analysis of Results There are a number of software programs available for the analysis of cell migration images. One of these is CellProfiler™ Cell Image Analysis Software offered free-of-charge by the Broad Institute*. You may find more information on this program online at www.cellprofiler.org. In order to analyze data from our Radius™ Cell Migration Assays, the CellProfiler™ software must be customized. For your convenience, we have developed add-ons that will customize the program for you. Please visit our website at www.cellbiolabs.com, type "Cellprofiler" in the Search box, and follow the instructions to download the appropriate add-on. *CellProfiler™ is a trademark of the Broad Institute. There is no relationship between Cell Biolabs, Inc. and the Broad Institute. Cell Biolabs offers these add-ons as a courtesy to our customers who wish to analyze data obtained using our Radius™ Cell Migration Assays.

Restrictions:	For Research Use only

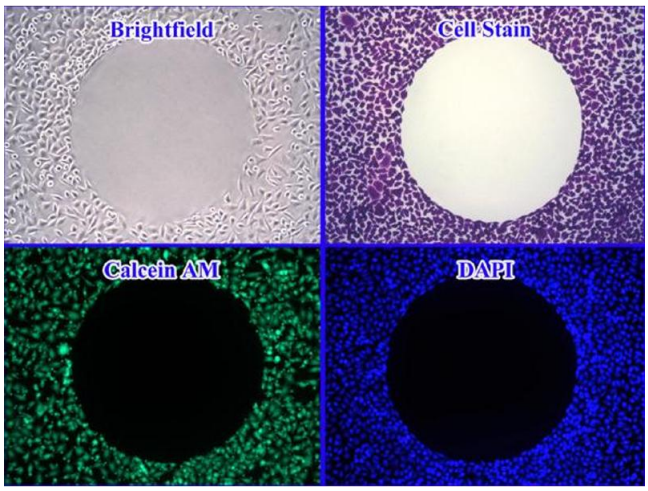
Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, aliquot and store the Radius™ Gel Removal Solution and DAPI Fluorescence Stain

Handling

at -20°C (avoid multiple freeze/thaw cycles), and transfer the Fixation Solution to 4°C. Store all other kit components at room temperature.

Images



Cellular Assay

Image 1. Various Detection Methods with Radius™ Cell Migration Assay. HeLa cells were seeded at 100,000 cells/well overnight. After removal of Radius™ Gel, cells were stained according to the assay protocol with Cell Stain Solution, Calcein AM (not included in kit), or DAPI.