

Cellomics[®] Cell Motility Kit

High-Content Screening Reagents

1815.2

Number	Description
K08-0001-1	Cell Motility Kit, sufficient materials for 5 × 96 wells
R02-0005-1	Cell Motility Kit, sufficient materials for 50 × 96 wells

Kit Contents:	K0800011	R0200051
Rhodamine Phalloidin	60 U	2 x 300 U
Blue Fluorescent Microspheres	5 x 2.0 ml	2 x 75 ml
Wash Buffer (10X)	100 ml	--
Permeabilization Buffer (10X)	100 ml	--
Thin Plate Seal Assembly	7/pack	--

Storage: Upon receipt store all kit components at 4°C. Keep the vials containing the Blue Fluorescent Microspheres and Rhodamine-Phalloidin solutions away from light. Allow buffers to warm to room temperature before use. See the **Solution Preparation** section for storage and stability of prepared solutions.

Warning: Please completely read these instructions and the accompanying material safety data sheets before using this product. The Cellomics Reagents are not for diagnostic use in humans or animals.

Introduction

The Cell Motility HCS Reagent Kit provides reagents and a protocol optimized for quantification of cell motility by directly measuring the size of tracks generated by migrating cells. The assay is performed on live cells growing on standard high density microplates. The kit includes Blue Fluorescent Beads, Rhodamine-conjugated Phalloidin for staining the cytoskeleton and assay buffers.

Cell motility is central to a number of biological and pathologic processes including cancer cell invasion and metastasis, inflammation, angiogenesis, wound repair, and embryonic development. The movement of cells occurs via the concerted activities of cell adhesion molecules, the actin cytoskeleton and an extensive network of signaling molecules. A combination of cell substrate adhesion, cell spreading, lamellar protrusion and directional control work together for net translocation. These activities are regulated both by external factors including extracellular matrix proteins, cytokines and soluble growth factors, and by intracellular signal transduction cascades.^{1,2}

The Cell Motility Kit facilitates the quantification of cell movement with images of fixed cells. This functional assay can predict the efficacy of potential drugs for a number of therapeutic areas. The assay is performed by plating cells on a lawn of microscopic fluorescent beads. As cells move across the lawn, they phagocytose and push aside the beads, clearing phagokinetic tracks behind them (Figure 1). The track area is proportional to the magnitude of cell movement. The reagents in this kit combined with the ArrayScan HCS Reader and the Cell Motility BioApplication Software enable automated plate handling, focusing, cell image acquisition, analysis and quantification. For a more detailed description of the image-processing algorithm, see the Cell Motility BioApplication Guide that accompanies the Cell Motility BioApplication software.

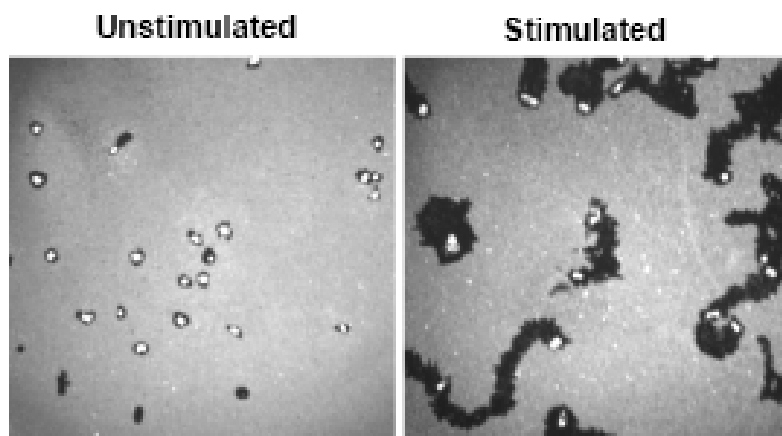


Figure 1. Tracks generated by L929 cells in the absence (unstimulated, left) or presence (stimulated, right) of serum. Cells within the tracks appear as intense fluorescent spots from concentrated beads internalized by the cells and the Rhodamine-Phalloidin that labels the cytoskeleton.

Additional Materials Required

- Methanol (Fisher, Product No. A408-1)
- Formaldehyde (37%) (Sigma, Product No. F1268)
- Collagen-I coated microplates; Recommended 96-well microplate for use on the ArrayScan HCS Reader: Falcon[®] Biocoat[®] (Fisher Scientific, Product No. 08-772-72)

Note: For the screening size kit, Wash Buffer and Permeabilization Buffer are available separately (please call customer service for more information).

Cell Preparation Information

- This protocol is optimized for L929 cells (ATCC, Product No. CCL-1).
- For cell culture use Minimum Essential Medium-Eagle (EMEM; BioWhittaker, Product No. 12-611Q) containing the following supplements (=EMEM Complete Medium): 10% fetal calf serum (BioWhittaker, Product No. 14-503F), 1X penicillin/streptomycin solution (BioWhittaker, Product No. 17-602E), 1% L-glutamine (BioWhittaker, Product No. 17-605E), 1% non-essential amino acids (BioWhittaker, Product No. 13-114E) and 1% sodium pyruvate (BioWhittaker, Product No. 13-115E).
- Split cells when they reach 60-80% confluency (every 2-3 days), diluting 1:5 to 1:10.
- For cell motility, harvest cells with trypsin-versene mixture (BioWhittaker, Product No. 17-161F) and dilute into EMEM Complete Medium (warmed to 37°C). For cells cultured in a T-75 flask, wash cells with 3 ml of trypsin. Add another 3 ml of trypsin and aspirate 2 ml. Allow cells to detach by incubating flask at 37°C for 5 minutes and then add 9 ml of EMEM Complete Medium and centrifuge cells at 400 × g for 5 minutes at room temperature.
- Wash 3X with Serum-Free Culture Medium (warmed to 37°C) (see **Procedure** section).
- Adjust cell density to 10⁴ cells/ml in EMEM serum-free medium and add 50 µl of the cell suspension to each well of a 96-well microplate (= 500 cells/well). Cell motility is stimulated with 50 µl of 10% fetal bovine serum (FBS) diluted in EMEM serum-free medium. See **Procedure** section.
- Incubate for desired time at 37°C in 5% CO₂ (18 hours for L929 cells).

Cell Motility Kit Protocol

A. Solution Preparation (per 96-well plate)

1X Wash Buffer	Add 20 ml of 10X Wash Buffer to 180 ml of ultrapure water. Store this solution at 4°C for up to 7 days.
1X Permeabilization Buffer	Add 2 ml of 10X Permeabilization Buffer to 18 ml ultrapure water. Store this solution at 4°C for up to 7 days.
Serum-Free Culture Medium	Prepare Cell Culture Medium with all supplements except FBS. Protocol requires 50 ml per plate. Prepare solution just before each assay and warm to room temperature before use.
Fixation Solution	Add 3.7 ml of 37% formaldehyde to 21.3 ml 1X Wash Buffer. Warm to 37°C until use. Prepare solution just before each assay.
Rhodamine-Phalloidin Stock Solution	Add 300 µl of methanol to the Rhodamine-Phalloidin tube. Mix well by vortexing. Store the stock solution for up to 12 months at -20°C desiccated and protected from light.
Staining Solution	Add 55 µl of Rhodamine-Phalloidin Stock Solution to 11 ml 1X Wash Buffer. Prepare solution just before each assay.

B. Procedure

Note: Use 100 µl per well unless indicated otherwise. The protocol requires ~2.5 hours post-compound incubation to perform.

- Suspend the Blue Fluorescent Microspheres (1 tube per 96-well plate) by vortexing for 30 seconds. Centrifuge the microspheres for 1 minute at 20,000 × g. The highest speed on a standard microcentrifuge is generally sufficient.
- Aspirate supernatant, add 0.5 ml 1X Wash Buffer and completely suspend beads by vortexing on maximum speed for at least 30 seconds.
- Centrifuge for 1 minute at 20,000 × g.
- Aspirate supernatant, add 0.5 ml 1X Wash Buffer and completely resuspend beads by vortexing on maximum speed for at least 30 seconds.
- Transfer the Blue Fluorescent Microsphere solution to a tube containing 7.5 ml 1X Wash Buffer, vortex on maximum speed for 60 seconds and immediately add 75 µl to each well of a 96-well plate coated with the desired substrate (For L929 cells, type 1 collagen).
- Incubate plate for 1 hour at 37°C in the dark.
- Wash plate five times with 200 µl of 1X Wash Buffer, removing each wash by aspiration. Leave buffer from last wash in wells. Plates may be stored in this format at 4°C for up to 1 week.
- Harvest L929 cells as described in Cell Preparation Section.
- Centrifuge cells at 400 × g for 5 minutes at room temperature. Resuspend cells in 10 ml Serum-Free Culture Medium, pre-warmed to 37°C. Washing the cells in the absence of serum is essential to prevent stimulation of cell motility.
- Repeat step 9 two more times and dilute cells to 10⁴ cells/ml in Serum-Free Culture Medium. Use 6 ml of the cell suspension per plate.
- Warm the Blue Fluorescent Microsphere plates to 37°C. Aspirate Wash Buffer from plates (as prepared in steps 1-7), and gently add 50 µl (5 × 10² cells) of the cell suspension to each well. Low cell seeding densities are important for minimizing overlapping of tracks. If test compound is known to prevent cell attachment, for best results pre-incubate cells at 37°C to let cells attach before motility stimulation/inhibition.
- Add 50 µl of Serum-Free Culture Medium containing supplements for negative controls or 50 µl of 10% FBS in EMEM Serum-Free Medium for positive controls (for a final concentration of 5% serum) to each well. Serum is a stimulator of L929 cell motility. When screening for cell motility antagonists, add test compounds before or during serum addition.
- Incubate for desired time (e.g., 18 hours for L929 cells) at 37°C in 5% CO₂.

14. Add 200 μ l of 5.5% warmed Fixation Solution to each well and incubate in fume hood at room temperature for 60 minutes. Do not remove medium before adding the Fixation Solution. Using warm Fixation Solution is critical to maintaining cell integrity.
15. Aspirate Fixation Solution and wash plate three times with 100 μ l of 1X Wash Buffer.
16. Aspirate Wash Buffer and add 100 μ l of 1X Permeabilization Buffer and incubate for 15 minutes at room temperature.
17. Aspirate Permeabilization Buffer, add 100 μ l Staining Solution and incubate for 30 minutes at room temperature.
18. Aspirate Staining Solution and wash wells three times with 100 μ l 1X Wash Buffer. Aspirate the last wash and fill wells with 200 μ l 1X Wash Buffer.
19. Seal plate and evaluate on the ArrayScan HCS Reader. Store sealed plates in the dark at 4°C.

Additional Information

A. Dose Response Curve and Motility

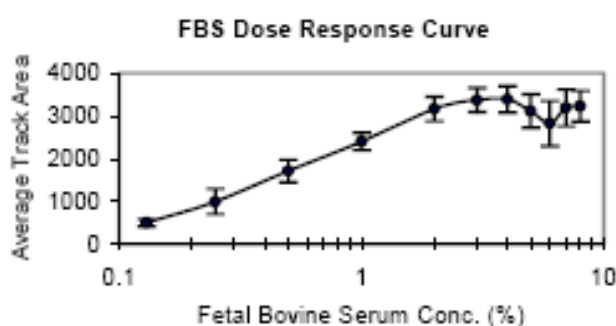


Figure 2. Dose response curve of cell motility stimulation in L929 cells. L929 cells were seeded into a 96-well plate coated with collagen I and Blue Fluorescent Beads, and incubated in the presence of increasing concentrations of fetal bovine serum. A dose-dependent stimulation of cell motility resulted. The average track area was quantified using the ArrayScan HCS Reader. Data are the mean from eight wells; error bars are standard deviations.

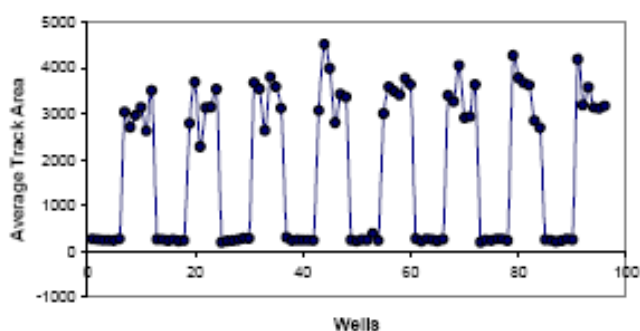


Figure 3. Track area of L929 cell motility of non-stimulated and stimulated cells. L929 cells were seeded into a 96-well plate coated with collagen I and Blue Fluorescent Beads. Columns 1-6 of the plate were negative controls treated with EMEM Serum-Free Medium. Columns 7-12 were positive controls treated with 10% FBS and incubated for 18 hours at 37°C. Stimulated L929 cells had track areas greater than 2,000 pixels and non-stimulated cells had tracks areas less than 300 pixels.

B. Microscope Information

Cells prepared and labeled according to these instructions can be used and analyzed by fluorescence microscopes using the appropriate filter set(s) or confocal microscopy. Optimization may be required when using slides, coverslips or multi-well chamber slides. Use image-processing software to quantify the targets. The approximate absorption/emission maxima of the fluorescent dyes are as follows:

Rhodamine Conjugates = 542/565 nm

Blue Fluorescent Beads = 365/415 nm

C. Recommendations for Automation

- **Plating Cells:** To improve the uniformity and throughput of plating cells, use a liquid handling system such as Thermo Scientific Multidrop[®] Combi or WellMate[®] Dispensers.
- **Dead Volumes:** Every piece of automation instrumentation has a non-recoverable dead volume associated with it. Be aware of these dead volumes, priming volumes and rinsing volumes when calculating your reagent requirements.
- **Nonspecific Binding:** Because of the potential of reagent interaction with large surface areas inherent to tubing, syringes and peristaltic pumps, pre-priming with reagents or pre-coating with protein blockers may be warranted.
- **Mixing:** Gentle mixing may be required when adding a DMSO-based solution to keep overly concentrated solutions from lying on top of the cell layer. Be careful not to dislodge cells or beads during mixing procedures.

- Cell Washing: Use an automated plate washer designed to gently wash attached cells. Be careful not to dislodge cells or beads during cell washing.
- Incubation: Minimize the time when plates with live cells are out of a controlled CO₂ environment. For best results, use an automated incubator to deliver plates to a pipetting deck.
- Exposure: Minimize operator exposure to fixative by some form of containment. Some reagents and compounds are light-sensitive; be aware of these constraints when scaling up for an automated run.
- Adapting to other plate formats: When using different plate types, adjust reagent volumes as needed. Some suggested starting volumes are listed in Table 1.

Table 1. Suggested volumes to use for different cell culture plates.

Kit Component	96-Well Plates	384-Well Plates	24-Well Plates
	(μ l/well)	(μ l/well)	(μ l/well)
Fixation Solution	100	25	400
1X Wash Buffer	100	25	400
1X Blocking Buffer	100	25	400
1X Permeabilization Buffer	100	25	400
Antibody Solution	50	12.5	200
Staining Solution	50	12.5	200
1X Wash Buffer (final wash)	150	37.5	200

Compatible BioApplication Software Modules

S50-0008-1

Cell Motility BioApplication

References

1. Klemke, R.L., *et al.* (1997). Regulation of cell motility by mitogen-activated protein kinase. *J Cell Biol* **137**:481-92.
2. Yujiri, T., *et al.* (2000). MEK kinase 1 gene disruption alters cell migration and c-Jun NH₂-terminal kinase regulation but does not cause a measurable defect in NF- κ B activation. *Proc Natl Acad Sci U S A.* **97**:7272-7.
3. Zhang, J.H., *et al.* (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* **4**:67-73.
4. Taylor, D.L., *et al.* (2007). High content screening: A powerful approach to systems cell biology and drug discovery. *Method Mol Biol* **356**. Humana Press, Totowa, N.J.

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