Cultrex™

Cell Migration Assay

Catalog Number: 3465-024-K (24-well Kit)
3465-096-K (96-well Kit)

Reagent kits for investigating chemotaxis.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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BACKGROUND

Cultrex® Cell Migration Assays were originally created in an effort to accelerate the screening process for compounds that influence chemotaxis, which is a fundamental component of angiogenesis (1), embryonic development (2), immune responses (3), and wound healing (4). This assay offers a flexible, standardized, and multi-well format for quantitating the degree to which cells migrate *in vitro* in response to chemoattractants in the presence of inhibitors or stimulants.

Cultrex Cell Migration Assays utilize a simplified Boyden chamber design with an 8 μm polyethylene terephthalate (PET) membrane. Ports within the migration chamber (top) allow access to the assay chamber (bottom). The inserts are sufficiently large to allow for subsequent analysis of migrating subpopulations, in response to chemokines, cytokines, drugs or other compounds of interest.

Detection of cell invasion is quantified using Calcein AM. Calcein AM is internalized, and intracellular esterases cleave the acetomethylester (AM) moiety to generate free Calcein. Free Calcein fluoresces brightly, and this fluorescence may be used to quantitate the number of cells that have migrated using a standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents with those from other lots or sources.
- Variations in sample collection, processing, and storage may cause sample value differences.

TECHNICAL HINTS

- When mixing or reconstituting solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

PRECAUTION

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Do not use past kit expiration date.

Note: *Components for this kit require different storage/shipping temperatures and will arrive in separate packaging*

CATALOG #	PART	PART #	AMOUNT PROVIDED	STORAGE OF UNOPENED MATERIAL
3465-024-K	25X Cell Wash Buffer	3455-096-04	2 vials (1.5 mL/vial)	Store at 2-8 °C.
	10X Cell Dissociation Solution	3455-096-05	2 vials (1.5 mL/vial)	
	Cell Invasion/Migration Plates, 24-well	3455-024-01	24-well plate	
	Calcein AM	4892-010-01	50 µg	Store at ≤ -20 °C.
3465-096-K	25X Cell Wash Buffer	3455-096-04	2 vials (1.5 mL/vial)	Store at 2-8 °C.
	10X Cell Dissociation Solution	3455-096-05	2 vials (1.5 mL/vial)	
	Cell Invasion/Migration Plates, 96-well	3455-096-01	96-well plate	
	Calcein AM	4892-010-01	50 µg	Store at ≤ -20 °C.

OTHER SUPPLIES REQUIRED

Reagents:

- Cell Harvesting Buffer (EDTA, trypsin, or other cell detachment buffer)
- Cell Culture Medium (as recommended by cell supplier)
- Serum-free Cell Culture Medium
- Chemoattractants or pharmacological agents for addition to culture medium
- Quenching Medium: Serum-Free Media with 5% BSA
- Sterile PBS or HBSS
- Distilled water
- Trypan blue or equivalent viability stain

Equipment:

- Pipettes and pipette tips
- 37 °C CO_2 incubator
- Low speed centrifuge and tubes for cell harvesting
- Hemocytometer or other means to count cells
- 50 and 500 mL graduated cylinders
- ≤ -20 °C and 2-8 °C storage
- Ice bucket
- Standard light microscope
- Timer
- Vortex mixer
- Fluorescent 96-well plate reader, top reader \(485 nm excitation, 520 nm emission)
- Clear, Flat bottom 24-Well Plates (if generating standard curve)

Disposables:

- 50 mL tubes
- Microcentrifuge tubes
- 1, 5, and 10 mL serological pipettes
- Gloves
- Hydrophobic coverslips (optional)
- Glass coverslips

REAGENT PREPARATION

1X Cell Wash Buffer - Dilute 3 mL of 25X Cell Wash Buffer in 72 mL of sterile, distilled water to make 1X Cell Wash Buffer.

1X Cell Dissociation Solution - Dilute 3 mL of 10X Cell Dissociation Solution in 27 mL of sterile, distilled water to make a 1X Cell Dissociation Solution.

Calcein AM Solution - Centrifuge microtube momentarily to pellet powder before opening tube, and add 30 µL of sterile DMSO to make working solution. Pipet up and down to mix, and store solution at < -20 °C **(do not foam)**.

ASSAY PROTOCOL

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

Cell Harvesting

Subject cells may be prepared for investigation as desired. The following procedure is suggested and may be optimized to suit the cell type(s) of interest.

1. Cells should be passaged 2 or 3 times prior to use in the assay, and adherent cells need to be 80% (but not 100%) confluent. Each chamber can accommodate 1×10^5 - 5×10^5 cells depending upon cell type. A 25 cm^2 or 75 cm^2 flask will yield approximately 3×10^6 or 9×10^6 cells, respectively. Plan to have enough cells for a standard curve, if used, controls and cell migration assay.

2. Starve cells by incubating 18-24 hours in Serum-free Cell Culture Medium prior to assay (0.5% FBS may be used if needed).

3. Prior to harvest, visually inspect cells, and record cell health, relative number, and morphology.

4. Wash cells two times with sterile PBS or HBSS. Use 5 mL per wash for a 25 cm^2 flask and 10 mL per wash for a 75 cm^2 flask.

5. Harvest cells. For 25 cm^2 flask or 75 cm^2 flask, add 1 mL or 2 mL, respectively, of Cell Harvesting Buffer, and incubate at 37 °C for 5-15 minutes (until cells have dissociated from bottom of flask).

6. Transfer cells to a 15 mL conical tube, and add 5 mL of Quenching Medium.

7. Centrifuge cells at 250 x g for 10 minutes to pellet, remove quenching medium, and resuspend cells in 2 mL of Serum-free Cell Culture Medium (0.5% FBS may be used if needed). Cells may need to be gently pipetted up and down with serological pipet to break up clumps.

8. Count cells, and dilute to 1×10^6 cells/mL in Serum-free Cell Culture Medium (0.5% FBS may be used if needed).

Conversion of Relative Fluorescence Units (RFU) into Cell Number

Many investigators express their results relative to untreated cells. In order to convert relative fluorescence units (RFU) into number of cells, standard curves are recommended. It is not necessary to use inserts to generate a standard curve. If used, a separate standard curve may be run for each cell type and assay condition. Control and experimental replicates should be performed in triplicate.

Standard Curve Determination

1. Your data should fall in the linear range of the curve. Determine the saturation range for your cells (*e.g.* 50,000-500,000 cells), beyond which, additional invasion would be difficult to detect because an increase in signal is no longer linear and approaches an asymptote.

2. Determine the total number of cells needed per standard curve (Table 1), and the required volume of medium.

3. Transfer volume of harvested cells needed to a 15 mL conical tube, and centrifuge at 250 x g for 10 minutes to pellet cells.

4. Remove supernatant, and resuspend cells in Cell Dissociation Solution at 1 x 10^6 cells/mL. (Phenol red in culture media will interfere with your signal).

5. Dilute cells for highest condition.

   a) 24-well Assay: for a final volume of 100 µL (*e.g.* 50,000 cells/100 µL) in 1X Cell Dissociation Solution, add 100 µL/well, and serially dilute remaining stock with 1X Cell Dissociation Solution to generate the number of cells needed in each well. Repeat dilutions until all conditions have been satisfied.

   b) 96-well Assay: for a final volume of 50 µL (*e.g.* 50,000 cells/50 µL) in 1X Cell Dissociation Solution, add 50 µL/well, and serially dilute remaining stock with 1X Cell Dissociation Solution to generate the number of cells needed in each well. Repeat dilutions until all conditions have been satisfied.

6. Prepare Calcein AM/Dissociation Solution:

   a) 24-well Assay - Add 12 µL of Calcein AM to 12 mL of 1X Cell Dissociation Solution. Invert to mix.

   b) 96-well Assay - Add 12 µL of Calcein AM to 5 mL of 1X Cell Dissociation Solution. Invert to mix.

7. Add 1X Calcein AM/Dissociation Solution to each set of wells containing decreasing numbers of cells, and incubate for one hour; omit cells from at least three wells to calculate background.

    a) 24-well Assay - Add 500 µL of Calcein AM/Dissociation Solution to each well.

    b) 96-well Assay - Add 50 µL of Calcein AM/Dissociation Solution to each well.

8. Read at 485 nm excitation, 520 nm emission (see Table 1 for sample data) to obtain RFU values.

9. Average your values for each condition; then subtract the background from each value (Table 1).

10. Plot standard curve RFU values vs. number of cells.

11. Insert a trend line (best fit) and use the equation $y = mx + b$ for each cell line to calculate the number of cells that invaded.

Table 1. Sample Data for Standard Curve (actual results may vary):

Average Cells/Well	Wells					
	1	2	3	Average	Background=254	
50,000	15,710	15,145	16,135	15663	- Bg. =	15409
25,000	9118	8702	8644	8821	- Bg. =	8567
10,000	4454	4257	4091	4267	- Bg. =	4013
5000	2609	2541	2599	2583	- Bg. =	2329
1000	930	922	881	911	- Bg. =	657

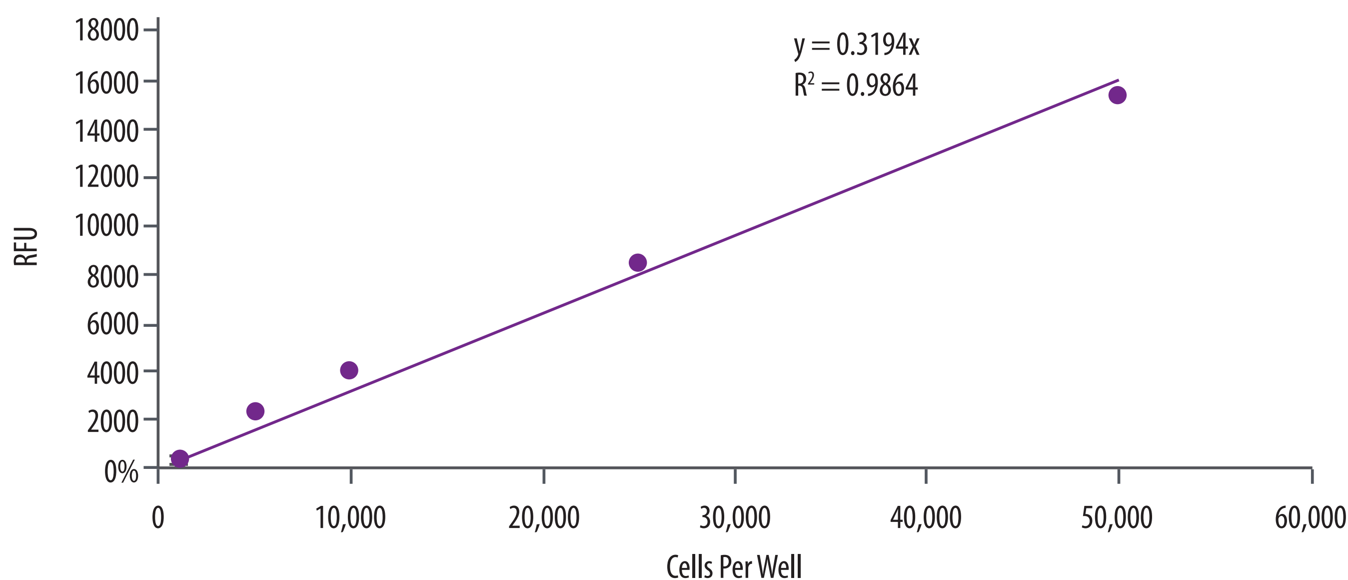

Figure 1. Standard Curve for a Cell Migration Assay. HT-1080 cells were harvested, diluted, incubated for one hour with Calcein AM, and assayed for fluorescence. The trend line and line equation are included on the graph. A separate standard curve for each tested cell line is recommended.

Cell Migration Assay

1. Culture cells to sufficient numbers to accommodate the pre-determined number of cells per chamber. Cells should be harvested at < 80% confluence.

2. 24 hours prior to assay, cells may need to be serum starved in order to allow ligands to bind to free receptors. This step may be omitted, depending on the cell types and ligands under investigation.

3. After 24 hours of serum starvation, if used, centrifuge cells at 250 x g for 10 minutes, remove supernatant, wash with 1X Cell Wash Buffer, count and resuspend at 1×10^6 cells/mL in a Serum-free Cell Culture Medium (0.5% FBS may be used if needed).

4. If desired, assay cells for standard curve; each cell line will require a separate standard curve.
5. Add cells into the top chamber for each well, with or without inhibitors/stimulants (100 µL/well for the 24-well Assay, 50 µL/well for the 96-well Assay). To compensate for background, omit cells from at least three wells.
6. Add Cell Culture Medium to bottom chambers (with or without drugs, chemokines, cytokines, etc.). Assemble chambers.

    a) 24-well assay: Add 500 µL/well

    b) 96-well assay: Add 150 µL/well

7. Incubate at 37 °C in CO_2 incubator; incubation times may be varied (4-48 hours).
8. After incubation, carefully aspirate top chamber, without puncturing the membrane, and wash each well with warm (37 °C) 1X Cell Wash Buffer.

    a) 24-well plate: 500 µL/well

    b) 96-well plate: 200 µL/well

9. Aspirate bottom chamber, and wash each well twice with 500 µL warm (37 °C) 1X Cell Wash Buffer.
10. Prepare Calcein AM/Dissociation Solution.

    a) 24-well Assay - Add 12 µL Calcein AM Solution to 12 mL of 1X Cell Dissociation Solution.

    b) 96-well Assay - Add 12 µL Calcein AM Solution to 10 mL of 1X Cell Dissociation Solution.

11. Add Calcein AM/Dissociation Solution to the bottom chamber of each well, reassemble the chambers, and incubate at 37 °C in a CO_2 incubator for 30 minutes.

    a) 24-well Assay: 500 µL/well of Calcein AM/Dissociation Solution.

    b) 96-well Assay: 100 µL/well of Calcein AM/Dissociation Solution.

12. Gently tap device 10 times on the side, and incubate at 37 °C in a CO_2 incubator for 30 minutes (one hour in total) for optimal dissociation.
13. Disassemble chambers (remove inserts), and read plate (assay chamber solutions/bottom) at 485 nm excitation, 520 nm emission using same parameters (time and gain) as standard curve(s), or controls.
14. Compare experimental data to controls, and convert RFU into cell number to determine the number of cells that have migrated, or failed to migrate according to experimental design.

DATA EXAMPLE

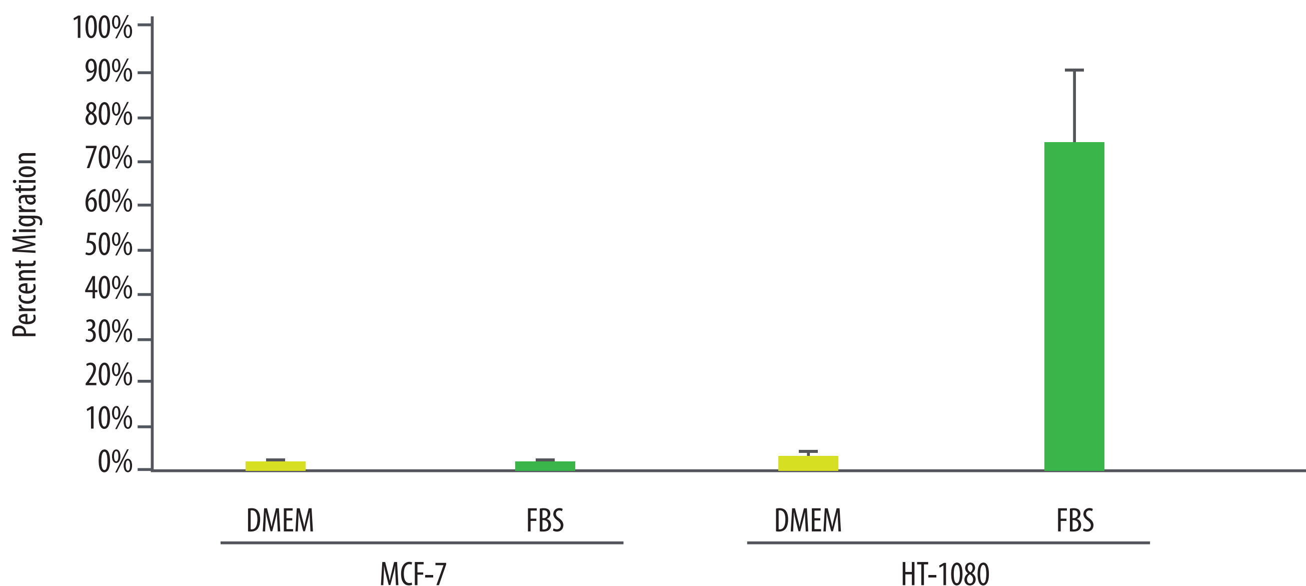

Figure 2. Results using MCF-7 and HT-1080 Cell Lines. The human fibroblastic cell line HT-1080 readily traverses through a perforated PET membrane with 8 µm pores in response to 10% FBS in the lower well whereas static MCF-7 cells do not migrate.

TROUBLESHOOTING

Problem	Cause	Action
High background	Insufficient Washing - agents in media, FBS, and/or chemo-attractant may react with Calcein AM.	Re-assay, and make sure to wash well.
		There is inherent variability in FBS from lot to lot; this can affect the assay if used.
	Contamination - proteases released by bacteria or mold may activate Calcein AM.	Start a new culture from seed stocks, and re-assay. If seed stock is contaminated, then it may be prudent to get new cells.
Well to well variability	Inconsistent pipetting.	Calibrate pipettors, and monitor pipet tips for air bubbles.
	Puncture membrane with pipet tips.	Disregard data from wells that are punctured; re-assay if necessary.
No signal	Cells did not migrate: Cell type may lack the needed receptor or chemoattractant may be insufficient or suboptimal.	Use early passage cells; check for cell surface receptor expression; run dilution series for chemoattractant of interest to determine optimal concentration.
	There is inherent FBS variability from lot to lot; this can affect assay if used.	Screening of different FBS lots may be necessary.
	Cells may have died as a result of treatment.	Test cells for viability in treatment regimen.

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07.20 3093465.2 12/20