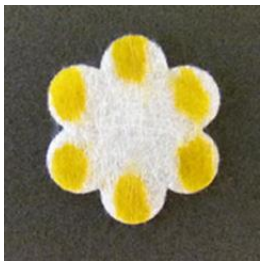
LENA BIOSCIENCES

Innovative 3D Cell Culture Tools for Life Sciences

GradientEZ INSTRUCTIONS



MATERIALS NEEDED:

a. For suspension cells: untreated 6-well plates, 12-well plates or Petri dishes
   For adherent cells: tissue culture treated 6-well plates, 12-well plates or Petri dishes
b. If placing GradientEZ into a Petri dish: autoclaved strips of filter or blotting paper
c. Sterile forceps
d. To cut out a circular section of GradientEZ and isolate cells from the center and petals or lobes: sterile scissors, a sterile circular biopsy punch, or a disposable Acuderm Accu-punch
   Punch diameter: 4 mm for SC-G001-0012, and 12 mm for SC-G002-0012
e. To quantify the number of migrated cells: CellTiter-Glo assay

INSTRUCTIONS

Step 1: Place the product in a sterile cell culture hood and open the package.

Step 2: Place GradientEZ into a Petri dish or a multiwell plate using sterile forceps.

Step 3: Pre-wet GradientEZ and aspirate the same volume prior to addition of cells.

A. Add DI water and let it spread (GradientEZ turns transparent/gray):
   - Add 70 ul to SC-G001-0012
   - Add 100 ul to SC-G002-0012

B. Touch GardientEZ surface using micropipette. Aspirate the same volume that was dispensed in A (GradientEZ turns white).
   If the GradientEZ does not turn white, there is still water in it. Press the GradientEZ surface again and aspirate.

Step 4: Addition of cells and reagents.

Migration and invasion assays:

- SC-G001-0012: add 0.2×10^5 – 1.0×10^6 cells to center and each petal
- SC-G001-0012: add 1.4×10^5 – 1.4×10^6 cells to center and half of that to each lobe

When using primary cells and for chemotaxis assays, these values can be lower.

For chemotaxis:

Depending on how you wish to setup a gradient you may either:

a. add cells to the center and chemoattractant to petals or lobes; or
b. add cells to petals or lobes and chemoattractant to the center.

Recommended volumes:

For SC-G001-0012: add 5 ul to center and 5 ul to each petal

For SC-G002-0012: add 20-25 ul to center and 10-12 ul to each lobe

After adding cells, allow few minutes for cells to settle and then add chemoattractant.

When adding to the center, *keep micropipette vertical and make sure to touch the center*.

Controls do not have chemoattractant.

For migration assays:

- If testing an inhibitor of cell migration you may either:
  a. add cells to the center and inhibitor(s) to petals or lobes; or
  b. add cells to petals or lobes and an inhibitor to the center.
- If testing an inducer of cell migration you may either:
  c. add cell and inducer to the center and let cells migrate to petals or lobes; or
  d. add cells with inducer to petals or lobes and let cells migrate to the center.
- If serum is used to stimulate migration, then:
  a. Remove serum from medium for 18-24 hours prior to addition of cells.
  b. Seed cells in a serum-free medium in the center and add serum-containing medium to petals or lobes.

Recommended volumes:

For SC-G001-0012: add 5 ul to center and 5 ul to each petal

For SC-G002-0012: add 20-25 ul to center and 10-12 ul to each lobe

After adding cells, allow 5 minutes for cells to adhere and then add modulators of cell migration.

When adding to the center, *make sure to touch the center*.

Controls do not have modulators of cell migration (e.g. inhibitors or inducers).

For invasion assays:

Seed cells that are suspended in the extracellular matrix at a lower matrix concentration than that in the invading region. For example, seed cells in 4 mg/mL Matrigel at the center and then add 8 mg/ml Matrigel to invading regions (petals or lobes). Alternatively, add cells in 4 mg/mL Matrigel to petals or lobes, and then add 8 mg/ml Matrigel to invading region in the center.

Recommended volumes:

For SC-G001-0012: add 6 ul to center and 6 ul to each petal

For SC-G002-0012: add 25 ul to center and 12 ul to each lobe

When adding to the center, *make sure to touch the center*.

After adding cells, place the plate or Petri dish in the incubator for 15-20 minutes for cells to attach (and Matrigel to gel) and then add medium.

For chemo-invasion assays:

Follow the protocol for invasion assay but add chemoattractant to the extracellular matrix in the invading region and *do not add medium*. (See step 6 to prevent drying).

For metastatic potential:

Seed tumor cells suspended in the extracellular matrix at the center. Then, seed other cell types suspended in the extracellular matrix in petals or lobes.

Recommended volumes:

For SC-G001-0012: add 6 ul to center and 6 ul to each petal

For SC-G002-0012: add 25 ul to center and 12 ul to each lobe

When adding to the center, *make sure to touch the center*.

After adding cells, place the plate or Petri dish in the incubator for 15-20 minutes for cells to attach (and Matrigel to gel) and then add medium. When *multiple cell types* are used, you may want to use minimum essential medium. For primary cells, you may want to omit serum.

Step 6: Prevention of evaporative losses.

- For cell invasion (but not chemo-invasion) assays, and when testing metastatic potential skip this step.

When cells and reagents are added, it is critical to fill the inter-wells, the regions between the wells of a multiwell plate, with DI water to prevent evaporative losses during cell taxis, migration of chemo-invasion. If using a Petri dish, place strips of autoclaved filter or blotting paper inside the dish (around GradientEZ without touching it), and wet with sterile DI water. Handle the strips using sterile forceps when placing into the dish.

Step 7: Place the dish or a multiwell plate in a humidified incubator.

Ensure that the water pan is full.

Incubate as long as you normally would in a Boyden-type chamber.

- If necessary, invasion assays and metastatic potential setup can be run for days since cells are incubated in the cell culture medium. If doing so, make sure to exchange medium as you normally would in a multiwell plate.

Step 8: Quantitative and qualitative characterization.

a. Qualitative time-lapse imaging

Requires a fluorescent microscope and fluorescently labeled cells. To prevent drying during imaging, see step 6.

b. Quantitative readout without cell isolation
    - Remove GradientEZ from the dish.
    - Cut out a circular section in the center using sterile scissors or punch.
    - Transfer the center, and petals or lobes to another 24-well plate and run CellTiter-Glo assay.
    - If high number of cells migrate or invade, you may also use alamarBlue assay (CellTiter-Blue assay) and/or lysed LDH assay. Lysed LDH assay is terminal.

c. Quantified number of migrated cells without cell isolation
    - Remove GradientEZ from the dish.
    - Cut out a circular section in the center using sterile scissors or punch.
    - Transfer the center, and petals or lobes to another 24-well plate and run CellTiter-Glo assay.
    - Generate a standard curve for CellTiter-Glo assay under the same incubation conditions that you will use in your migration/invasion assay, prior to the assay.
    - If high number of cells migrate or invade, you may also use alamarBlue assay (CellTiter-Blue assay) and/or lysed LDH assay. Lysed LDH assay is terminal.

d. Quantitative readout with cell isolation
    - Remove GradientEZ from the dish.
    - Cut out circular section in the center using sterile scissors or punch.
    - Transfer the center, and petals or lobes to another 24-well plate.
    - Cell isolation:
        - For chemotaxis and cell migration assays follow protocol on Page 11 of SeedEZ cell recovery protocol to isolate cells. Note that SeedEZ and GradientEZ share the same material. For isolation using conical tubes follow protocol on Page 8.
        - For invasion and chemo-invasion assays follow protocol on Page 22 of SeedEZ cell recovery protocol to isolate cells. Note that SeedEZ and GradientEZ share the same material. For isolation using conical tubes follow protocol on Page 14.
    - Use a hand-held cell counter, or count using hemocytometer. For multiple cell types, use FACS, when possible.