GRADIENTECH

Version: 2014-09-01

Protocol for chemotaxis of human blood neutrophils in CellDirector® 2D

Preparation of cells

1. Equilibrate serum-free media (e.g. RPMI-1640 from Gibco®) overnight at 37 °C, to avoid bubble formation during the experiment.
2. Spin your neutrophil suspension at 1000 rpm for 5 min.
3. Pre-activate the neutrophils by resuspending them in 50 ng/ml IL-8, diluted in RPMI-1640. Leave the cells to activate during 5 min at 37 °C.
4. Spin your cell suspension at 1000 rpm for 5 min.
5. Resuspend the neutrophils in RPMI-1640 to a final concentration of 0.25 x 10^6 cells/ml.

Cell Loading

1. Inject 200 µl of the cell suspension into CellDirector 2D with a pipett (slowly).
2. Incubate the CellDirector 2D assay with the green side facing up in a 10 cm Ø Petri dish for 45 min at 37 °C. Check that the cells have adhered by looking in a microscope. The CellDirector assay can remain within the Petri dish when using the inverted microscope.
3. Fill syringe 1 with RPMI-1640. Fill syringe 2 with RPMI-1640 containing your chemoattractant.
4. Start the CellDirector 2D experiment as described in the manual.
5. Take a note of the orientation of the CellDirector assay and on which side the chemoattractant is put on.
6. Collect images using bright-field microscopy (10x objective) at 2 fpm (1 image every 30 s).

We thank Dr Francois Binet at the Dep. of Medical Biochemistry and Molecular Cell Biology, Uppsala University, Sweden, for sharing the protocol.