GRADIENTECH

PROTOCOL

Protocol for chemotaxis of human monocyte cell line U937 using CellDirector® 2D

CELLS AND REAGENTS: U937 cells (histiocytic lymphoma origin)
RPMI1640 medium containing 10% FBS
Poly-L-lysine

EQUILIBRATE ALL MEDIA IN THE INCUBATOR ONE NIGHT BEFORE USE

Pre-coating CellDirector 2D surface

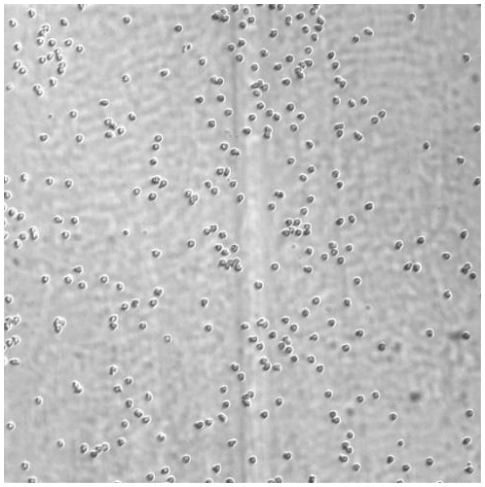
1. Inject 200µl of poly-L-lysine (50µg/ml) into CellDirector 2D. Pre-coat overnight in an incubator at 37°C with 5% CO_2 supply. Keep CellDirector 2D, with the green side facing up, on a petri dish containing a wetted tissue to prevent evaporation.
2. Next day, inject 2 x 200µl of RPMI medium into CellDirector 2D to wash it twice and block the surface (leave 200µl of medium in the device) for 30 min in the incubator.

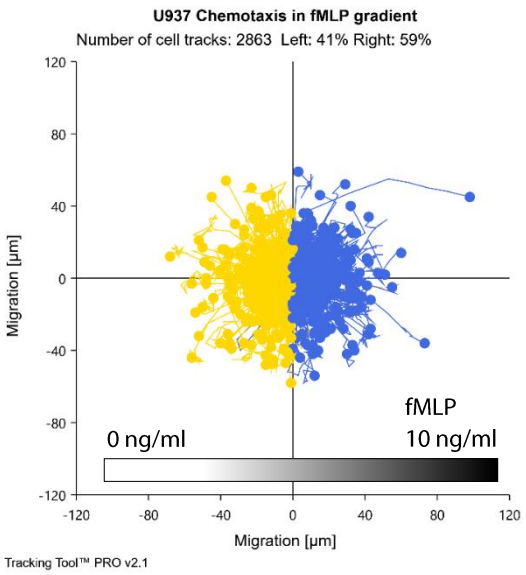
Preparation of U937 cells

1. Culture U937 cells in RPMI medium containing 10% FBS and 1% Penicillin in an incubator at 37°C with 5% CO_2 supply.
2. Collect cultured U937 cells and centrifuge at 1500 rpm for 5 mins. Resuspend cells to a concentration of $2x10^6$ cells/ml.

Loading cells into CellDirector 2D and induce chemotaxis towards fMLP

1. Inject 200µl U937 cell suspension into CellDirector 2D.
2. Incubate for 1 hour in the incubator and check the adherence of cells under the microscope.
3. Prepare the syringes by filling one with 1ml of chemoattractant (e.g. 10ng/mL of fMLP in RPMI) and the other syringe with RPMI medium as control.
4. Set the syringes to the pump and assemble CellDirector 2D to the holder on your microscope.
5. Start the CellDirector® 2D experiment as described in the *Short User Guide*. See also the *Guide for Flow Directions and Gradient Orientation*.
6. Use an initial flow of 5µl/min to remove unattached cells and then change the speed to 1µl/min.
7. Collect images for 2 hours using bright field microscopy (10x objective) at 0.5 fpm (1 image every 2 minutes). Preferably, collect images at serial ROI along the gradient channel, as well as from the two control channels.
8. Analyse using Tracking Tool™ PRO software (www.gradientech.se).





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