GRADIENTECH

PROTOCOL
Human T-lymphocyte chemotaxis towards a gradient of CXCL 12 using CellDirector® 2D

CELLS AND REAGENTS NEEDED:
- Jurkat cells
- PBS (Phosphate-buffered saline)
- RPMI 1640 medium, GlutaMAX™ & fatty acid free BSA (0.1%) supplemented
- Fibronectin
- CXCL 12 (C-X-C motif chemokine 12)
- FBS (Fetal Bovine Serum)

Preparation of Jurkat

1. Culture Jurkat cells in RPMI medium supplemented with 10% FBS at 37°C in a humid atmosphere with 5% CO_2.
2. Wash cells twice with RPMI medium (free from FBS)
3. Resuspend to a final density 1 x10 ^6 cells /ml in supplemented RPMI medium

Coating of CellDirector® 2D

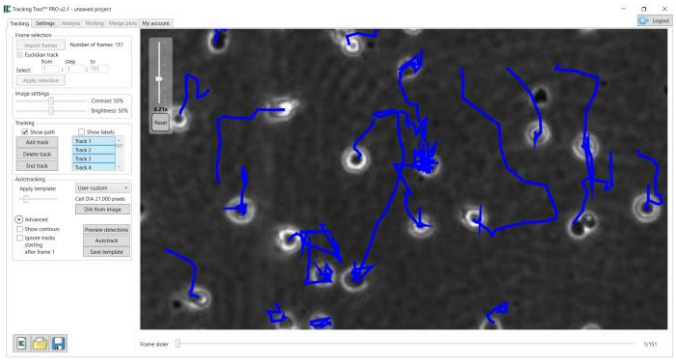
1. Add 200 µl fibronectin (10µg/ml in PBS) into an empty CellDirector® 2D
2. Put the CellDirector assay, with the green side facing up in a 10 cm Ø Petri dish and leave in room temperature for an hour.
3. Temperature equilibrate the CellDirector in the incubator for 20 mins.

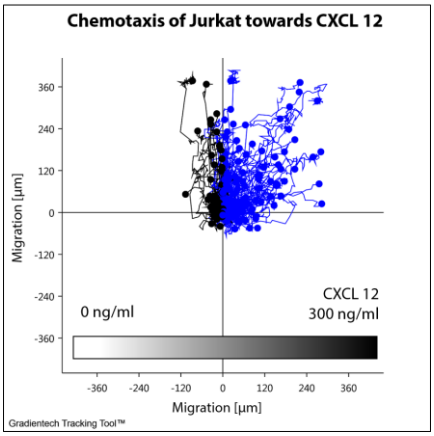
Load Jurkat into CellDirector® 2D and induce chemotaxis towards CXCL 12

1. Pipet 2 x 200 µl of the Jurkat suspension into the CellDirector® 2D coated with fibronectin.
2. Let Jurkat to adhere (approx. 3 hours) by putting the CellDirector® 2D assay, with the green side facing up, in a petri dish containing a wet tissue. Place in a humidified incubator, 37C. *Note: Check that cells have adhered by looking in a microscope*
3. Prepare the syringes by filling one syringe with supplemented RPMI medium and the second syringe with CXCL 12 [300 ng/ml] in supplemented RPMI medium. *Optional: incubate the syringes in the incubator some time before filling them with medium, so everything is at the same temperature.*
4. Start the CellDirector® 2D experiment as described in the *Short User Guide*. See also the *Guide for Flow Directions and Gradient Orientation*.
5. Collect time-lapse images using bright-field microscopy (10x objective) at 2 fpm (1 image every 30 second). Preferably, collect images at serial ROI along the gradient channel, as well as from the two control channels.
6. Analyse using Tracking Tool™ PRO software (www.gradientech.se).

RESULTS:

Total number of tracked cells	254
Cells migrating towards increasing CXCL 12	70%
Average Velocity [µm/min]	2.1
Directionality	0.32





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