GRADIENTECH

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Protocol for murine B-lymphocytes cell migration in CellDirector® 2D

Make sure that all solutions including the cell media, the coating solutions and the PBS that the coating is diluted in, are equilibrated overnight in a 37 °C cell incubator, to avoid any bubble formation. Remember to put the equilibrated solutions back in the incubator shortly after use.

According to this protocol, the B-lymphocytes are not subjected to any pre-activation step, as it has been noticed that pre-activation with potent activators like PMA can impair the cells ability of chemotaxis.

Collagen I, gelatin, poly-L-ornithine and Matrigel™ coatings have been tested, but do not show as good cell adherence results as fibronectin.

CellDirector2D coating

1. Add 200 µl of fibronectin (16 µg/ml in PBS) to an empty CellDirector2D.
2. Put the CellDirector assay, with the green side facing up in a 10 cm Ø Petri dish and incubate 1h in the cell incubator at 37 °C.

Preparation of cells and cell loading

1. Start with resuspending 10 million cells in 250 µl equilibrated RPMI medium.
   Murine B-lymphocytes are normally in suspension and only a fraction will adhere. The cell concentration needs to be optimized depending on how big fraction of your cells that adhere well.
2. Add 200 µl of your cell suspension to the coated CellDirector assay (pipet slowly). Let the cells adhere in the cell incubator for 1h. Check that the desired number of cells have adhered by looking in a microscope. The CellDirector assay can remain within the Petri dish when using the inverted microscope.
3. Prepare equilibrated RPMI medium (control) and medium with 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).

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