

CIM device cell staining protocol.

It is recommended when performing pilot experiments to stain the cells on the underside of the membrane to get an impression about the extent of cell migration and its comparison with CI signal.

To stain the cells:

- a. Disassemble the CIM plate by pressing the two handles on the bottom chamber (BC) and gently remove the top chamber (TC).
- b. Remove media from wells of the TC with a multi-channel pipette while avoiding contact with the membrane.

Stain the migrated cells on the CIM membrane.

The Diff-Quick staining kit by Fisher Scientific is recommended (Cat No: NC9979740); however, any typical cell stain used in Boyden assays will work.

The modified staining procedure for Diff-Quick is briefly described below:

- Place the TC into fixative solution for 2 min with membrane side containing the electrodes in direct contact with the fixative.
- Place the TC into solution I for 1 min with membrane side containing the electrode in direct contact with the solution.
- Place the TC into solution II for 1 min with membrane side containing the electrode in direct contact with the solution.
- Gently rinse the TC with water.
- Remove unmigrated cells from top of the TC by gently wiping with a Q-tip or similar type of cotton swab inside the well.
- Visualize and count the cells under an upright microscope.