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Cell migration assay or Transwell assay

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- 1 Cells are washed in cold PBS, detached with 5mM EDTA, centrifuged at 1200 g for 7 min and resuspended in serum-free medium.
- 2 Transwell chambers (Corning Costar) are prepared filling with 600 µl of complete medium the lower chamber.
- 3 The desired amount of cells is plated in 100 µl of serum-free medium in the upper insert (8.0 µm pore size) of each Transwell chamber and incubated at 37°C.
- 4 After 24 hours at 37°C, fill with 600 µl of 5mM EDTA an empty well beside each used Transwell chamber.
- 5 Eliminate DMEM from the upper insert and move it inside an EDTA well.
- 6 Collect DMEM from the lower chamber and put some EDTA inside, to detach cells possibly fallen from the upper insert.
- 7 Detach cells migrated on the underside of the upper insert using a plastic scraper and collecting them in the EDTA well.
- 8 Mix in one tube cells collected from the lower chamber and scraped from the insert and centrifuge at 1200 g for 7 min, to count the migrated cells.