

Podosome reformation assay – Linder lab

Podosome Disruption

This protocol is performed according to (Linder et al. 2000), with just a few modifications.

Before drug treatment, starve the cells in medium without serum at least for 3 hours, even better if overnight.

After starvation, treat the cells with 25 μ M tyrosine kinase inhibitor PP2 (add the right amount of drug directly to the starvation medium) at least for 15 min. At this point, most of the cells (>70-80 %) don't have podosomes anymore (or just show few of them).

Podosome Reformation

To study podosome reformation, carefully aspirate the starvation medium with PP2 (wash out step) and feed the cells with normal complete medium. If you are handling cells on coverslips, it is better to carefully transfer them into new wells containing fresh complete medium, instead of aspirating the medium, since a strong vacuum pump might cause detachment of some cells.

Initial formation of podosomes can now be observed as early as 5 min. Usually, 30-45 min after the wash out step, most of the cells again show podosomes in comparable numbers to the untreated condition.