Actin Polymerisation

Please note – this protocol has been optimised for certain conditions and is meant as a guide only, you may need to alter some parameters to suit your own experiment

1. Serum-starve cell culture in media containing 0.5% FCS for 2 hours prior to start of assay
2. Wash cells twice with pre-warmed PBS
3. Stimulate with chemoattractant at optimal concentration. Incubate for 15 minutes, mixing a few times during incubation
4. Fix sample in 3.7% PFA for 10 minutes at RT (avoid any methanol-containing fixatives as methanol can disrupt actin during the fixation process)
5. Pellet cells and wash twice with PBS
6. Permeabilise cells using 0.1% TritonX for 3-5 minutes on ice
7. Wash twice with PBS
8. Incubate cells with 50 μl of NBD-phallacidin (5 μl stock in 200 μl PBS) for 30 minutes on ice
9. Wash twice with PBS
10. Resuspend in 300 μl for analysis on cytometer (NBD detected in FL1)