Immunfluorescense

1. Trypsinating and counting cells.
2. Seeding of 2 x 10^4 cells/well (ibidi® cell culture dishes) and incubate for 24h at 37°C and 5% CO_2 atmosphere
3. After 24 h cells were washed once with 300µl PBS
4. Incubating cells for 20 minutes with 4% PFA (Paraformaldehyde) and washing with 300µl PBS
5. Incubating cells for 10 minutes with 0,5% Triton-PBS and washing with 300µl PBS
6. Incubating cells for 30 minutes with 1% BSA and washing with 300µl PBS
7. Incubating cells with primary antibody (Anti-Flag® M1 Monoclonal Antibody from SIGMA) at 4°C over night
8. Incubating cells with secondary antibody (Pacific Blue™ goat anti-mouse IgG (H+L) from *life technologies*) for 2h at room temperature
9. Wash three times with PBS/PBS-0,5%-Triton (300µl)
10. Images were captured with Leica DMI 6000 B