Immunofluorescence: General PBMC Protocol

The following protocol used human PBMC that were isolated from whole human blood (Ficoll method) and plated onto fibronectin-coated coverslips in 10% FCS/DMEM supplemented with pen/strep and L-glutamine. Cells were allowed to adhere at 37˚C for 1 hour before being fixed in 4% paraformaldehyde for 20 minutes at room temperature.

1. Fixed cells were permeabilized with 0.1% Triton X-100/PBS for 2 minutes before being washed extensively with PBS.
2. Coverslips were blocked with 1% BSA/PBS.
3. Slides were stained with the primary antibody (diluted in 1%BSA/PBS) at room temperature for 1 hour.
4. Cells were stained with the secondary antibodies for cytoskeleton and nuclei detection.
5. Coverslips were mounted onto glass slides with medium and imaged.