Immunofluorescent protocol

All IF staining in the Human Protein Atlas project is performed using a standard protocol as described below.

Cell cultivation

96 well glass bottom plates are coated with 40 µl 12.5 µg/ml fibronectin (diluted in 1xPBS) for 1 h in RT or longer at 4°C.

Cells are seeded at concentrations varying between 8 000-25 000 cells/well, and grown 6-24 h depending on cell line.

Fixation & Permeabilization

The cells are washed once with 1xPBS and then fixated for 15 min in ice cold 4% PFA (diluted in cell culture media supplemented with 10% FBS).

Permeabilization for 3x5 min with 0.01% Triton X-100.

Antibody staining

After washing with 1xPBS, the cells are incubated with the primary antibodies O/N at 4°C.

The cells are washed 4x10 min with 1xPBS before secondary antibodies are added and incubated for 1.5 h at RT.

The secondary antibodies are removed and DAPI (1.15 µM) is added for 5 min.

The cells are washed 4x10 min with 1xPBS before the plate is mounted with glycerol in 10xPBS and sealed.

Primary antibodies

Primary antibodies are diluted in 1xPBS supplemented with 4% FBS.

HPA antibody, diluted to 2 µg/ml

Anti-alphatubulin, ab7291 Abcam, 1:1000

Anti-KDEL, ab50601 Abcam, 1:400

Secondary antibodies

Secondary antibodies are diluted in 1xPBS supplemented with 4% FBS.

Alexa Fluor 647, A-21247 ThermoFisher Scientific, 1:400

Alexa Fluor 555, A-21424 ThermoFisher Scientific, 1:800

Alexa Fluor 488, A-11034 ThermoFisher Scientific, 1:800