

Immunofluorescence (ICC-IF) protocol

ICC-IF stainings in the Human Protein Atlas project are performed using a standardized protocol, as described below.

Cell cultivation

96-well flat glass bottom plates are coated with 12.5 µg/ml fibronectin (diluted in 1xPBS) for 1 h at RT, or longer at 4°C. The coating solution is removed just prior to seeding.

Cells are seeded at concentrations varying between 5 000-25 000 cells/well, and grown for 18-24 h at 37°C with 5% CO₂, depending on cell line.

Fixation & Permeabilization

The cells are washed once with 1xPBS and then immediately fixed for 15 min in ice cold 4% PFA (diluted in 1xPBS supplemented with 10% FBS). A few stainings have been performed using fixation in methanol for 3x5 min.

The cells are permeabilized for 3x5 min with 0.01% Triton X-100.

Antibody staining

The cells are washed once with 1xPBS and then incubated with primary antibodies O/N at 4°C.

The cells are washed 4x10 min with 1xPBS and then incubated with secondary antibodies 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 wells are mounted with glycerol containing 1xPBS and sealed.

Primary antibodies

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

HPA antibody, diluted to 2 µg/ml (unless otherwise indicated)

Anti-alpha tubulin, Abcam ab7291, 1:1000

Anti-calreticulin, Abcam ab2908, 1:800 (previously anti-KDEL, Abcam ab50601, 1:400)

Secondary antibodies

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

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

Goat-anti-chicken Alexa Fluor 647, ThermoFisher Scientific A-21449, 1:800

Note: For each gene, the use of PFA or methanol for fixation, as well as the dilution factor for each HPA antibody, is stated under the Antibodies and Validation page on the Human protein Atlas.