Atlas Antibodies AB
Phone:+46-8 54 59 58 50
E-mail: contact@atlasantibodies.com
www.atlasantibodies.com

Immunofluorescence Protocol

Please see if a specific Atlas antibody is verified for immunofluorescence under "Tested Applications" in the Product Datasheet.The Product Datasheet is viewed by clicking on the product name or product number in our online store; atlasantibodies.com/store/list.

A large number of the antibodies have been used in subcellular localization studies by immunofluorescence (IF) staining of three cell lines; A-431, U-2 OS and U-251 MG. Each cell line is stained with a primary Atlas antibody, two organelle probes specific for the endoplasmatic reticulum and micro-tubules, as well as counterstained with the nuclear probe DAPI. The IF images can be viewed on the Human Protein Atlas web portal (proteinatlas.org).

Reagents

Primary antibodies:

- Atlas antibodies at a working concentration of 1-4 µg/ml.

NOTE 1: *The dilution of the primary antibody is to be considered as a guideline only. Optimal dilution must be determined by the user.*

- Chicken anti-Calreticulin polyclonal antibody (Abcam, Cambridge, UK) diluted 1000x.

- Mouse anti-alpha Tubulin monoclonal antibody (Abcam, Cambridge, UK) diluted 1000x.

Secondary antibodies:

- Alexa® Fluor 555 goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA) diluted 800x.

- Alexa® Fluor 647 goat anti-chicken IgG (Invitrogen, Carlsbad, CA, USA) diluted 800x.

- Alexa® Fluor 488 goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) diluted 800x.

Protocol

Sample preparation
All washes are performed at room temperature.
1. A multiwell, glass-bottomed, plate (Whatman, Maidstone, UK) is coated with fibronectin (concentration 12.5 µg/ml) for 1 hour at room temperature.
2. Cells are seeded (10.000-15.000 cells per well) and incubated at 37°C in humified air with 5.2% CO_2, for 4 hours.

Immunostaining
3. Growth medium is removed and the cells are washed in PBS (8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2).
4. The cells are fixed for 15 minutes in ice cold 4% paraformaldehyde pH 7.2-7.3 in growth medium supplemented with 10% fetal bovine serum (FBS).
5. The cells are permeabilized 3x5 minutes with 0.1% Triton X-100 in PBS.
6. The cells are washed with PBS and incubated overnight at 4°C with primary antibodies in PBS supplemented with 4% FBS.
7. The following day the cells are washed 4x10 minutes with PBS and incubated for 1.5 hours in room temperature with secondary antibodies in PBS supplemented with 4% FBS.

NOTE 2: *The secondary antibodies are fluorescently labeled and thus light sensitive. The sample should be kept in dim light in this as well as in the following steps.*

8. The cells are counterstained for 4 minutes with the nuclear stain DAPI (Invitrogen, Carlsbad, CA, USA) 0.6 µM in PBS.
9. The cells are washed 4x10 minutes with PBS and mounted in glycerol + 10% 10xPBS.

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Warranty: The products supplied by Atlas Antibodies AB are warranted to meet stated product specifications and to confirm to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Atlas Antibodies AB's instructions. Atlas Antibodies AB's sole liability is limited to replacement of the product or refund of the purchase price. All products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. No products from Atlas Antibodies AB may be resold, modified for resale or used to manufacture commercial products without prior written approval from Atlas Antibodies AB.
Atlas Antibodies AB, AlbaNova University Center, SE-106 91 Stockholm, Sweden. Phone: +46-8-54 59 58 50, Fax: +46-8-54 59 58 51
E-mail: contact@atlasantibodies.com, Web: www.atlasantibodies.com.