HPA Cell Atlas Standard Immunostaining Protocol

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dx.doi.org/10.17504/protocols.io.x2dfqa6

Human Protein Atlas

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ABSTRACT

The Cell Atlas, part of the Human Protein Atlas project, systematically investigate the spatiotemporal subcellular distribution of human proteins. This is our standard protocol for indirect immunostaining of cells cultured in 96-well glass bottom plates.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pubmed/19896565

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

All volumes in the protocol refer to volume per well in a 96-well microplate. Unspecified incubation temperatures are at room temperature. Immunostaining can be performed manually or with the help of a liquid handelling robot.

MATERIALS

NAME	CATALOG #	VENDOR
Disodium phosphate	S7907	Sigma Aldrich
Monopotassium phosphate	P9791	Sigma Aldrich

MATERIALS TEXT

Buffers are prepared using MilliQ water

10X PBS

160 g NaCl
4 g KCl
28.8g Na_2HPO_4
4.8 g KH_2PO_4
Dissolve in 1600 ml Milli-Q. Adjust pH to 7.2 and add Milli-Q up to two liters. Filter and autoclave the buffer.

4% PFA/1X PBS

Use lab coat, eye protection and gloves and work in a fume hood.

1. Pre-heat 150 ml 10% FBS/1X PBS in a 250 ml beaker to 60°C on a hot plate with magnetic stirrer.
2. Add 50 ml 16% paraformaldehyde to the warm PBS and leave stirring for 20 min at 60°C.
3. Add concentrated NaOH until the solution reaches pH ~11.
4. Allow the solution too cool down to room temperature.
5. Set pH to 7.2-7.3 using first concentrated HCl, and then diluted HCl for fine adjustment.
6. Aliquot and store at -20ºC. Thaw at room temperature right before use.

DAPI

1. Dissolve 10 mg of DAPI powder in 2 ml MilliQ water to a 5 mg/ml (14.3 mM) concentrated stock solution. Store at -20°C.

2. Mix 80 µl 5 mg/ml DAPI stock solution with 920 µl MilliQ water to a pre-diluted 400 µg/ml DAPI solution. Aliquot and store at -20°C.

Glycerol/10X PBS

1. Add 5 ml 10X PBS to 45 ml of glycerol. Mix.
2. Autoclave or filter sterilize.

SAFETY WARNINGS

Paraformaldehyde (PFA) is harmful in contact with skin or if inhaled, causes severe skin burns and eye damage, may cause an allergic skin reaction, may cause respiratory irritation, is suspected of causing genetic defects and may cause cancer.

BEFORE STARTING

Cells are grown over night in 80 ul cell culture media (according to providers specifications) in 96-well glass bottom microplates coated with fibronectin (12.5 ug/ml). Optimal seeding concentration/confluency upon staining varies between cell lines and need to be tested in each setting.

Prior to immunostaining, primary antibodies generated within the Human Protein Atlas (HPA) project are diluted to 2 ug/ml in blocking buffer (4% FBS/1XPBS) together with 1 ug/ml tubulin marker (mouse anti-alpha tubulin, Ab7291, Abcam) and 1.25 ug/ml ER marker (chicken anti-calreticulin, Ab2908, Abcam).

Fixation

1 Remove the growth medium (i.e. aspirate ~ **80 µl** from all wells).

2 Wash the cells once with **40 µl** 1X PBS.

3 Fix the cells by incubating in **40 µl** 4% PFA/1XPBS for 15 minutes. **00:15:00**

Permeabilization

4 Remove the PFA and permeabilize the cells by incubating with **40 µl** 0.1 % Triton X-100/1X PBS for 3 x 5 minutes. **00:05:00** **00:05:00** **05:00:00**

5 Remove the Triton X-100 and wash the cells once with **40 µl** 1XPBS.

Primary Antibody Incubation

6 Add **40 µl** blocking buffer with diluted primary antibodies; 2 ug/ml HPA antibody, 1 ug/ml anti-alpha tubulin (Abcam, Ab7291), and 1.25 ug/ml anti-calreticulin (Abcam, Ab2908). Incubate over night at 4°C
or for two hours at room temperature.

4 °C **16:00:00 overnight**

or

20 °C (RT) **02:00:00**

Secondary Antibody Incubation

Secondary Antibody Incubation

7 Remove the blocking buffer with primary antibodies and wash the cells with 40 µl 1X PBS for 4 x 10 minutes. 00:10:00 00:10:00 00:10:00 00:10:00

8 Add 40 µl blocking buffer with diluted secondary antibodies; 2.5 ug/ml anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific, A11034), 2.5 ug/ml anti-mouse Alexa Fluor 555 (Thermo Fisher Scientific, A21424), and 2.5 ug/ml anti-chicken Alexa Fluor 647 (Thermo Fisher Scientific, A21449). Incubate at room temperature for 90 minutes while keeping dark. 01:30:00

DAPI Nuclear Staining and Mounting

9 Remove the blocking buffer and incubate with 40 µl 0.2 ug/ml DAPI/1X PBS for 10 minutes. 00:10:00

10 Wash the cells with 40 µl 1X PBS for 4 x 10 minutes. 00:10:00 00:10:00 00:10:00 00:10:00

11 Fill the wells with glycerol/10X PBS and seal the plate with an adhesive aluminium PCR plate seal. Plates can be stored at 4°C for at least two weeks.