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Immunocytochemistry of autophagosome-associated proteins

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We use this protocol and it's working

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Abstract

Protocol for immunocytochemistry used on primary cortical neurons in Palumbos et al., 2025. Protocol can be adapted to other cultured cell types and antibodies. Described protocol works for autophagosome associtated proteins

Fix Cells

1 Heat 4% paraformaldehyde (PFA) to 37 °C

2 Add 4% sucrose by volume

3 Wash cultured cells 2X with warmed 1X PBS

4 Fix with 4% PFA + sucrose for 00:08:00 at 37 °C

5 Wash with 1X PBS for 5 minutes

6 Repeat wash 2X go to step #5

7 Stopping point - store at 4°C in 1X PBS or continue

Permeabilize Cells

8 Add permeabilizing agent and incubate cells.

Note

Permeabilization method should be determined based on protein(s) to be detected.
Follow Step 8.2 (MeOH permeabilization) for autophagosome or lysosome associated proteins
Follow Step 8.3 (Triton-X permeabilization) for other visualization
*You may need to try both methods to find best fit for a given antibody

8.1 MeOH permeabilization - Add 1 mL cold (-20°C) pure MeOH to well and place in -20°C for 8 minutes.

8.2 Triton X permeabilization - Add 0.2% Triton X-100 in PBS to well for 15 minutes at room temperature (not ideal for autophagosomes or lysosomes).

9 Wash 3X for 5 minutes with 1X PBS.

Block Cells

10 Prepare block (5% goat serum, 1% BSA, + sodium azide) in advance. Filter sterilize and keep at 4°C.

11 Add block to plates and incubate for 01:00:00 at Room temperature .

Primary Antibody Incubation

12 Prepare primary antibody dilution in block (1:250 good starting dilution if unknown).

13 Incubate overnight with primary antibody at 4°C.

Secondary Antibody Incubation

14 Wash 3X for 5 minutes with 1X PBS.

15 Prepare secondary antibody dilution in block (1:1000 good starting dilution).

16 Incubate at Room temperature for 02:00:00 .

17 Wash 3X for 5 minutes with 1X PBS.

18 Add Hoechst 1:1000 for 10 minutes.

19 Wash 3X for 5 minutes with 1X PBS.

20 Mount coverslip for imaging.