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Immunocytochemistry

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Michael Henderson[1], Naman Vatsa[1]

[1]Van Andel Institute

ASAP

Lindsay Meyerdirk
Van Andel Institute

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is a protocol for immunocytochemistry for 24 and 96 well cell culture plates.

Materials

	A	B	C	D
	Vendor	**Catalog#**	**Qty**	**Description**
	RPI	A30075-100.0	1	Bovine Serum Albumin
	Electron Microscopy Services	15713	10 X 10ml	20% Paraformaldehyde

Preparation

1 Prepare 1x phosphate buffer saline (PBS)

2 Prepare fixation solution: 4% paraformaldehyde/4% sucrose in PBS

> **Note**
>
> can be stores at 4°C for up to 1 month

3 Prewarm the fixative solution to 37 degrees Celsius

4 Prepare blocking buffer: 3% Bovine Serum Albumin (BSA) in PBS
- Seal the conical with parafilm to prevent evaporation and store at 4 degrees Celsius

5 Prepare 10% stock of Triton-X-100 in PBS

6 Prepare permeabilization buffer: 0.3% TX-100 in 3% BSA in PBS

Staining

7 Thoroughly aspirate media from each well without disturbing cells

8 Add pre-warmed fixative solution to the wells and incubate at room temperature for 15 minutes. (0.5 mL/well for 24-well plate, 100µL/well for 96-well plate).

9 Rinse 5X with PBS. This and subsequent washes can be done with the plate washer for 96-well plates.

10 Permeabilize neurons with a permeabilization buffer for 15 minutes at room temperature.

11 Rinse 3X with PBS.

12 Block neurons for a further 50 minutes with a blocking buffer at room temperature.

13 Dilute primary antibodies in blocking buffer.

14 Incubate cells with primary antibody for 2 hours at room temperature or overnight at 4°C.

15 Rinse 5X with PBS.

16 Dilute Alexa Fluor conjugated secondary antibodies corresponding to primary antibodies in blocking buffer in 1:500 dilution

17 Incubate cells in secondary antibodies for 1 hour at room temperature.

> **Note**
>
> Make sure that the secondary antibodies will not recognize each other or crossreact with the same primary antibodies!

18 Rinse 5X with PBS.

19 Mount coverslips onto glass slides with Prolong Gold mounting media. 96-well should be incubated with 100 µL DAPI 1:10,000 in PBS.

20 Seal the 96-well plate with a black seal.

21 Visualize staining on a fluorescent microscope.