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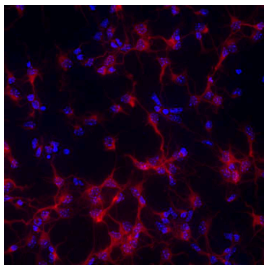
Immunofluorescence for Primary Brain Cell Cultures



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

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Abstract

Here we describe a protocol to detect specific proteins by immunofluorescence on cells cultured as monolayers. The protocol has been tested on primary cortical neurons and primary astrocytes isolated from mouse cortical tissues.

Guidelines

This protocol has been tested for primary astrocytes and cortical neurons cultured in 4- or 8-well chambers slides.

For astrocytes, coat slides with Poly-D, L-lysine hydrobromide (PDLL) (Sigma #P9011) for 1 hour at 37C and then wash twice with H2O.

For cortical neurons, coat with Poly-l-ornithine: Cat. #: P3655, Sigma and Laminin: Cat. #: L2020, Sigma for 2 hours at 37C. Perform 2 quick rinses with 1X PBS. Do not let the slides dry. Keep the slides well submerged in 1X PBS at RT until plating. Aspirate PBS and perform 2 quick rinses with ddH2O right before plating.

Materials

	A	B	C
	Name	**Company**	**Catalog**
	Prolong Glass antifade Mountant	Thermo Fisher Scientific	P36984
	Formaldehyde 16%	Thermo Fisher Scientific	28906
	Superblocking Buffer 100%	Thermo Fisher Scientific	37515
	Tween-20	Millipore Sigma	P1379
	Triton X-100	Millipore Sigma	T9284
	Hoechst	Thermo Fisher Scientific	H3570
	Poly-l-ornithine:	Millipore Sigma	P3655
	Laminin	Millipore Sigma	L2020
	Poly-D, L-lysine hydrobromide (PDLL)	Millipore Sigma	P9011
	Millicell EZ SLIDE 4-well glass, sterile	Millipore Sigma	PEZGS0416
	Slip-Rite Cover Glass	Thermo Fisher Scientific	152250

BUFFERS:

-Coating buffer in PBS
Poly-D, L-lysine hydrobromide (PDLL) (Sigma #P9011)
Dissolve 5mg in 500ml PBS (0.01mg/ml)

Poly-l-ornithine: 20mg/mL; For usage, dilute to 20µg/mL in ddH2O (1:1000)
Laminin: 1mg/mL; For usage, dilute to 5µg/mL in ddH2O (1:200)

-Fixation buffer
4% Formaldehyde in PBS

- Permeabilization Buffer
0.3% Triton X-100 in PBS

-Antibody hybridization buffer in PBS
10% Superblocking buffer
0.1% Tween-20

-Nuclear staining buffer
Hoechst 33342 1mg/ml solution in water

Day 1: Fixation, Permeabilization, Blocking, Primary Antibody

3h 30m

1 Fixation. Aspirate culture media and wash once with ice-cold 1X PBS. Add 4% Formaldehyde fixation buffer. Incubate at room temperature (RT) for 15 min. Use an orbital shaker using low speed.

2 Wash 3 times with ice-cold PBS. Incubate for 5 min on the orbital shaker at RT
Wash 1 ☐
Wash 2 ☐
Wash 3 ☐

3 Permeabilization: Add 500uL of 0.3% Triton in PBS per well (for 8-wells, reduce to 250uL), incubate at RT 5 min on the orbital shaker

4 3X Washes: Ice-cold PBS for 5 min (Incubate at RT, Orbital shaker)
Wash 1 ☐
Wash 2 ☐
Wash 3 ☐

5 Blocking: Add 600uL of 100% Superblocking Buffer. Incubate at RT for 1h with orbital shaker

6 Primary antibody hybridization: Dilute antibody in antibody hybridization buffer. Incubate o/n at 4°C or 1 hour at RT using an orbital shaker
Suggested volumes: 600µL/well for 4-wells & 300µL/well for 8-wells

Day 2: Secondary antibody

2h

7 Remove Primary Ab hybridization buffer. Antibodies can be stored at 4C and re-used.

8 3X Washes: Ice-cold PBS for 5 min (Incubate at RT, Orbital shaker)
Wash 1 ☐
Wash 2 ☐
Wash 3 ☐

9 Secondary antibody hybridization: Dilute secondary antibody in antibody hybridization buffer. Incubate at RT for 1 hr using an orbital shaker.
Suggested volumes: 500µL per well for a 4-well slide

Secondary antibodies are coupled with fluorophores. Perform these steps in the dark using dark chambers

10 3X Washes: Ice-cold PBS for 5 min (Incubate at RT, Orbital shaker)
Wash 1 ☐
Wash 2 ☐
Wash 3 ☐

11 Stain every well with Hoechst 33342 diluted 1:1000 in PBS. Incubate at RT for 3 min on an orbital shaker

12 3X Washes: Ice-cold PBS for 5 min (Incubate at RT, Orbital shaker)
Wash 1 ☐
Wash 2 ☐
Wash 3 ☐

Day 2: Slides Mounting

30m

13 Remove silicone gaskets from the glass slides. With a Kimwipe, remove the excess of PBS without touching the surface with the cells

14 Spread ~200uL of Prolong Glass antifade mounting medium across the whole slide. Slowly cover the glass slide with a glass coverslip and avoid making air bubbles. Seal the coverslip to the slide with clear nail polish

15 Let the slides dry for 24 hr at RT in the dark before the fluorescent microscopy analysis image. For long-term storage, keep the slides at 4°C in dark chambers.