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Immunocytochemistry

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We use this protocol and it's working

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Abstract

This protocol describes how to perform immunocytochemistry on fixed cells.

Fixing

1 The cells were washed three times with 1xPBS (Thermo Fisher Scientific) and fixed for 15-20 minutes with 4% paraformaldehyde, followed by two washes with 1xPBS. Note: Add 4% (w/v) PFA, enough to cover the cells well (e.g., 100µl to a well in a 96-well plate and 200µl to a well in a 48-well plate) and incubate for 15-20 min. Avoid drying the wells while washing.

Blocking and permeabilization

1h

2 Non-specific antibody binding sites were blocked using PBS^{+} (PBS containing 0,1% Triton-X; Thermo Fisher Scientific: HFH10) supplemented with 5% normal goat serum (NGS; Thermo Fisher Scientific: 31872) for 01:00:00 at Room temperature

1h

Staining

2h

3 Subsequent incubation with **primary antibodies** diluted in PBS^{+} with 1%NGS was performed Overnight at 4 °C

1h

	Antibody target	Provider	Cat# Number	Dilution
	LMX1	Merck Millipore	Cat# AB10533	1:1000
	FOXA2	R&D Systems	Cat# AF2400	1:1000
	OTX2	R&D Systems	Cat# AF1979	1:1000
	EN1	Novo Nordisk	NA	1:500
	TH	Merck Millipore	Cat# AB152	1:1000
	MAP2	Merck Millipore	Cat# AB5622	1:400
	IBA-1	AbCam	Cat# ab5076	1:500
	PU.1	Cell Signaling	Cat# 2258S	1:300
	CD45	Bio Legend	Cat# 304002	1:300

4 The next day (Day 2), the primary antibody was removed, and cells were washed three times with 1XPBS. Note: Avoid drying the wells while washing.

5 Cells were incubated with appropriate Alexa 488 and Cy3-conjugated secondary antibodies and DAPI (1mg/ml; 1:1000) for 01:00:00 at Room temperature, protected from light. Note: Add the 2° antibody-containing solution to the cells (~120 µl/cm²), wrap the plate or chamber slides in aluminium foil to avoid bleaching of the fluorophores, and incubate for 1 h at room temperature (RT) on a shaker. Note: 2° antibody dilution needs to be optimised based on the vendor it's purchased from, we used 1:400 for both Alexa 488 and Cy3-conjugated secondary antibodies. 1h

6 Wash the cells three times in PBS, leave the stained cells in PBS in the well, and leave the plate wrapped in aluminium foil at 4°C until microscopic analysis. Note: Use PBS containing 0.02% sodium azide (NaN3) to prevent microbial growth. Note: Avoid drying the wells while washing

7 Cells were washed three times with 1XPBS. Stained cells on chamber slides or mounted on glass slides were imaged using a Leica SP8 confocal laser scanning microscope (CLSM).