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Immunocytochemistry (ICC) for Progenitor markers FOXA2 and OTX2

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

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Abstract

This is the protocol for Immunocytochemistry (ICC) for Progenitor markers FOXA2 and OTX2.

Attachments

DOCX

Immunofluorescence p...

17KB

Materials

	A	B	C
	Blocking Buffer (BP)	1x PBS 5% BSA 0.3% Triton X-100	To make 10mL of Blocking Buffer: 0.5g of BSA 30µl of Triton X-100 Fill to 10mL with 1x PBS
	1º antibody (-20ºC fridge)	**FOXA2 Rb OTX2 Mus IgG**	
	2º antibodies (4ºC fridge)	**A11008 Alexa Flour 488** goat anti-rabbit IgG (HHH+L) **A10036 Alexa Flour546** donkey anti-mouse IgG (H+L)	Thermo Fisher Cat # A11008 Thermo Fisher Cat # A10036
	DAPI (-20ºC fridge)	**Stock 1mg/mL**	Final (1µg/mL)
	- Each well of both 8µm Chamber slide and 48-well plate should use 200µL of solution		

1 Grow the cells on either 8µm chamber slide or 48-well plate. (1×105cells/well)
- Coating and plating densities can be found in the differentiation kit

2 Aspirate medium and rinse with 1x PBS

3 Fixation: Cover cells with warm 4% PFA and fix for 20 min at RT.
- Cover the vessel and place in the hood

4 Rinse 3X in 1x PBS for 5 min each at the lab bench

5 Blocking and permeabilize for 30 min at RT with Blocking Buffer

6 1º antibody incubation dilute in 1/3 blocking buffer + 1x PBS and incubate overnight in the cold room
-20º (box labelled antibodies for iPSCs)
- FOXA2 Rb à (1:250)
- OTX2 Mus IgG à (1:250)
o Add 4µl of antibody per 1mL of prepared antibody solution

7 Rinse three times in 1x PBS for 5 min each

8 2º antibody incubation for 1hr at RT in the dark
- A11008 Alexa Flour 488 goat anti-rabbit IgG (HHH+L) 1:400 in PBS
- A10036 Alexa Flour546 donkey anti-mouse IgG (H+L) 1:400 in PBS
o Add 2.5µL of antibody per 1mL of prepared antibody solution
∗Combine these two antibodies in one tube. Ex. If you were making 1.6mL (for 8 wells) you would add 4µL of each antibody

9 Rinse 3X in 1X PBS for 5 min each

10 Incubate with DAPI at (1µg/mL) for 10min at RT (Stock is 1mg/mL which is 1000x)
a. Add 1µl of Stock DAPI to 1mL of prepared solution.

11 11. Rinse 2X in 1X PBS and image.