



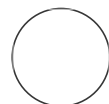
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# 🌐 Immunocytochemistry (ICC) for Progenitor markers FOXA2 and OTX2

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OPEN ACCESS



## ABSTRACT

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

## ATTACHMENTS

[Immunofluorescence protocol for iPSCs Fp markers .docx](#)

**DOI:**  
[dx.doi.org/10.17504/protocols.io.q26g7ppp3gwz/v1](https://dx.doi.org/10.17504/protocols.io.q26g7ppp3gwz/v1)

## MATERIALS

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**protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.q26g7ppp3gwz/v1>

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

**Created:** Aug 16, 2023

A	B	C
<b>Blocking Buffer (BP)</b>	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
<b>1° antibody (-20°C fridge)</b>	<b>FOXA2 Rb OTX2 Mus IgG</b>	
<b>2° antibodies (4°C fridge)</b>	<b>A11008 Alexa Flour 488</b> goat anti-rabbit IgG (HHH+L) <b>A10036 Alexa Flour546</b> donkey anti-mouse IgG (H+L)	Thermo Fisher Cat # A11008 Thermo Fisher Cat # A10036
<b>DAPI (-20°C fridge)</b>	<b>Stock 1mg/mL</b>	Final (1µg/mL)
- Each well of both 8µm Chamber slide and 48-well plate should use 200µL of solution		

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86555

**Keywords:** ASAPCRN

- 1** Grow the cells on either 8µm chamber slide or 48-well plate. (1x10<sup>5</sup>cells/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** 1° antibody incubation dilute in 1/3 blocking buffer + 1x PBS and incubate overnight in the cold room
  - 20° (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** 2° 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.