**Immunocytochemistry (ICC) for Progenitor markers FOXA2 and OTX2**

|  |  |  |
| --- | --- | --- |
| **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. (1x105cells/well)

* **Coating and plating densities can be found in the differentiation kit**

1. Aspirate medium and rinse with 1x PBS
2. Fixation: Cover cells with warm 4% PFA and fix for 20 min at RT.

* **Cover the vessel and place in the hood**

1. Rinse 3X in 1x PBS for 5 min each at the lab bench
2. Blocking and permeabilize for 30 min at RT with Blocking Buffer
3. 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 🡪 (1:250)**
* **OTX2 Mus IgG 🡪 (1:250)** 
  + **Add 4µl of antibody per 1mL of prepared antibody solution**

1. Rinse three times in 1x PBS for 5 min each
2. 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
  + **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

1. Rinse 3X in 1X PBS for 5 min each
2. Incubate with DAPI at (1µg/mL) for 10min at RT (Stock is 1mg/mL which is 1000x)
   1. **Add 1µl of Stock DAPI to 1mL of prepared solution.**
3. Rinse 2X in 1X PBS and image.