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Immunocytochemistry Protocol for Progenitor Cells in 96-Well Plates

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Ernest Arenas: Deceased 15.09.2024

SOX6 mDA differentiation

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

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Abstract

This protocol was used to tag canonical markers with fluorophores on *in vitro* differentiated KOLF2.1J cells.

Troubleshooting

Cell Seeding

1 Seed ~300,000 progenitor cells per well in a 96-well plate.

2 Culture as needed before fixation.

Fixation

37m

3 Remove medium from each well.

2m

4 Add 4% PFA to each well. Incubate for 20 minutes at 4°C.

20m

5 Wash wells 3× with PBS to remove residual fixative (5 minutes each wash).

15m

Permeabilization & Blocking

1h

6 Prepare blocking/permeabilization buffer:
- 5% normal donkey serum
- 0.3% Triton X-100 in PBS (PBST)

7 Add buffer to each well. Incubate for 1 hour at Room temperature

1h

Primary Antibody Incubation

16h

8 Dilute **primary antibodies** in PBST + 1% donkey serum. *Remember to mix antibodies with hosts that will not interact undesiredly with secondary antibodies. Example: goat primary antibody with goat secondary antibodies.*

9 Add to each well. Incubate **overnight at 4°C**. Overnight 4 °C

16h

Secondary Antibody Incubation

45m

10 Wash wells 3× with PBST (5 minutes each wash at room temperature). 15m

11 Add **Alexa Fluor-conjugated secondary antibodies** (e.g., Alexa488, Alexa555, or Alexa647) diluted in PBST. Incubate for **30 minutes at room temperature**, protected from light. Room temperature 00:30:00 . *Remember not to mix secondary antibodies that will interact with each other, example goat anti-donkey and donkey anti-rabbit.* 30m

Nuclear Staining

15m

12 Add **DAPI** to each well. Incubate for **15 minutes** at room temperature, **protected from light**. 15m

13 Wash **3× with PBS (5 minutes each)**. 15m

Imaging

14 Store samples in PBS (short term, <48h) or fluorescence mounting media (mid-long term).

15 Image using a fluorescence microscope (appropriate channels for Alexa dyes and DAPI).