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ICC Staining of Fixed Organoid Cells for Flow Analysis

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Katharina Meyer[1]

[1]Wyss Institute

Katharina Meyer
Wyss Institute

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We use this protocol and it's working

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Abstract

Indirect ICC Staining of PFA Fixed Dissociated Organoid Cells for Flow Analysis

Dissociation of Organoids

1 Wash organoids in each well with PBS 2 times.

2 Transfer organoids into a 2-ml Eppendorf tube containing 300-500 µl of cold Cell Recovery Solution for Matrigel embedded organoids (Corning).

3 Incubate on ice for 1 hour.

4 Spin down at 1000g for 1 minute.

5 Remove supernatant and wash with 1 ml cold PBS once.

6 Incubate organoids with 300-500 µl cold Accutase for 15 minutes.

7 Spin down at 1000g for 1 minute.

8 Remove supernatant and wash with cold PBS once.

9 Dissociate and resuspend the cells in 500 µl cold 4% PFA to start fixation.

10 Incubate on ice for 1 hour.

11 Spin down at 3000g for 1 minute.

12 Remove supernatant and wash with 3 × 1 ml PBS. Resuspend pellet each time by vortexing.

13 Keep cells in PBS until permeabilization.

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14 Recover cells by spinning down at 3000g for 1 minute.

15 Count cells.

16 Remove supernatant and resuspend the pellet with 300 µl blocking buffer (3% Normal donkey serum and 0.3% Triton X-100 in PBS).

17 Incubate for 30 minutes and briefly vortex the cells at 30% speed.

18 Spin down at 3000g for 1 minute.

19 Remove supernatant and resuspend the cells with blocking buffer + primary antibody/ies (100-250 µl and minimum of 100K cells) via vortexing at 70% speed.

20 Lay all tubes on a rack holder and secure them with tape.

21 Incubate on a shaker at 4°C overnight.

22 Wash the cells 3 × 500 µl PBS. Spin down at 3000g for 1 minute.

23 Resuspend the cells with 100-250 µl of blocking buffer + secondary antibody/ies.

24 Incubate the cells at room temperature for 1 hour and briefly vortex the cells at 30% speed.

25 Wash the cells 3 × 500 µl PBS. Spin down at 3000g for 1 minute.

26 Resuspend the cells in 0.5-1 ml PBS and filter the cells through a mesh of 0.45 µm. Keep the cells from light. On ice.