Immunofluorescence Staining of Organoids

Materials:
8-Well μ-Slide 80826
Fluoromount-G™ mounting medium 0100-01
Triton™ X-100 T8787
DAPI D1306
PBS 10010056
4% PFA J19943K2
Bovine Serum Albumin (BSA) 9998

Permeabilisation Buffer: 1X PBS / 0.2% Triton™ X-100
Blocking Buffer: 1X PBS / 1% BSA / 0.3% Triton™ X-100

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1. Culture organoids in 8-well μ-Slides.
2. Remove culture media and wash organoids with 200 µL PBS at room temperature for 5 min.
3. Remove PBS and fix cells with 4 % PFA for 30 min at 4 °C, with (orbital) shaking at around 450 rpm.
4. Remove PFA (take care not to lose organoids).
5. Repeat PBS wash three times, 5 min each, with shaking at 45 rpm on rocker.
   Note: this step is critical for the removal of Matrigel.
5. Add 200 µL Permeabilisation Buffer and incubate for 30 min at room temperature.
6. Add 200 µL Blocking Buffer and incubate for 30 min at room temperature. Prepare primary antibodies during the incubation.
7. Add 200 µL primary antibody diluted in Blocking Buffer, and incubate for 2 hours at room temperature or overnight at 4 °C.
8. Repeat PBS wash three times. Prepare secondary antibodies during the washes.
9. Add 200 µL secondary antibodies and DAPI diluted in Blocking Buffer, leave for 1 hr at room temperature.
   Note: shield cells from light using foil from now on.
10. Repeat PBS wash three times.
11. Wash the cells in ddH_2O.
12. Mount the cells in Fluoromount-G™ mounting medium, leave at room temperature until dry, and store slide at 4 °C prior to imaging.