Immunofluorescence Staining for Hepatocytes in Collagen Sandwich Culture

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Solutions and Materials

1x PBS
4% PFA in PBS

Blocking solution
10% horse serum in PBS

Antibody solution
5% horse serum in PBS

TNT solution
10 mM Tris-HCl pH 8.0
300 mM NaCl
0.1 % Tween

0.1% Triton X-100 solution
0.1% Triton X-100 in PBS

Method
For this method, the primary hepatocyte sandwich culture has to be cultured on sterile glass cover slips.

All steps are carried out at room temperature (RT) if not indicated otherwise.

- Remove the medium of the cells and wash once with PBS
- Fix the cells with 4% PFA for 30 min
- Add 1x PBS. *Cells can be stored like this at 4 °C.*
- Add 0.1 % Triton X-100 solution for at least 1 h
- Block with blocking solution for 1 h
- Suck 5 holes into the collagen layer using a vacuum pump
  This step is essential – otherwise the antibodies will not pass through the collagen layer
- Immediately add the primary antibody in antibody solution
- Incubate overnight on a shaker
- Wash for 2-4 h with TNT solution or overnight at 4 °C
- Add the secondary antibody in antibody solution for 5-6 h at 37 °C or overnight at 4 °C
- Wash several times with TNT solution
- Leave cells overnight in TNT solution at 4 °C
- Wash with dest. water
- Mount the samples with 8 µl Mowiol on glass slide

- Dry them overnight at RT or for 1-2 h at 37 °C in the dark
- Store the coverslips at 4 °C