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Emir's methanol fixation IF protocol

Emir Bora Akmeriç¹¹MDC Berlin

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protocol .

AG Gerhardt



Emir Bora Akmeriç

Methanol fixation protocol for immunofluorescence, should hopefully work for Claudin-5 staining.

Emir Bora Akmeriç 2021. Emir's methanol fixation IF protocol. [protocols.io](https://protocols.io/view/emir-39-s-methanol-fixation-if-protocol-b2bzqap6)
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Methanol is a toxic substance, work under the hood at all times!

Fixation 45m

- 1 Bring methanol to -20C and prepare compartment in cell culture freezer to quickly transfer slides/dishes. Get a box of ice **-20 °C** 5m
[Methanol P212121 Catalog #PA-33900HPLCCS4L](#)
- 2 Incubate sample in methanol for **12 minutes**. 12m
- 3 Wash samples with PBS **3 times on ice** 5m

4 Incubate samples with PBS on ice for **5 minutes, repeat 3 times** 18m

5 Store samples in the fridge until further use ⚠ **4 °C**

Blocking/Staining 19h 33m

6 Block for 1hr in 5% normal goat serum diluted in PBS 1h

7 Make primary antibody mix for the next step in the meantime

8 Incubate primary antibody diluted in blocking buffer **overnight** at 4C. 16h

9 Wash samples with PBS **3 times** 5m


10 Incubate samples with PBS for 5 minutes, **repeat 3 times** 18m

11 Incubate with secondary antibody diluted to 1:1000 in blocking buffer for 2h 2h

Mounting 30m

12 Wash samples with PBS **3 times** 5m

13 Incubate samples with PBS for 5 minutes, **repeat 3 times** 17m

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- 14 Add 1:100 DAPI diluted in PBS, incubate for 10 minutes 10m
 - 15 Wash samples with PBS 2 **times**
 - 16 Add mounting media, probably PBS in this case or mowiol mixed with 10% dabco