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# Immunocytochemistry (ICC)

mineechoi[1,2]

[1]Queen Square UCL Institute of Neurology; [2]The Francis Crick Institute



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Minee-Liane Choi
UCL Institute of Neurology, The Francis Crick Institute

ABSTRACT

This protocol describes how to do immunocytostochemistry for primary and hiPSC-derived cells.

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## Cell fixation

1 Cells are fixed in [M]**4 % volume** paraformaldehyde (PFA) and stored in phosphate-buffered saline (PBS) until use.

## Permeabilizing and blocking cells

2 Wash cells with PBS twice.

3 Incubate the cells in [M]**0.2 % volume** Triton X-100, [M]**5 % volume** bovine serum albumin (BSA) for **01:00:00** at **Room temperature** . 1h

[M]**5 % volume** BSA (made in PBS) is used to block non-specific binding.

For ATTO 425 labelled Aptamer staining, cells are permeabilized with [M]**0.25 % volume** Triton X-100 and blocked with [M]**10 % volume** normal goat serum (NGS) for **00:20:00** followed by another **03:00:00** with [M]**0.1 % volume** Trion X-100 and [M]**10 % volume** NGS.

## Incubate cells in primary antibodies

4 2h

Do not wash after permeabilising and blocking steps

Dilute primary antibody in [M]**5 % volume** BSA and incubate cells at **4 °C** , **Overnight** or **01:00:00** at **Room temperature** .

The final volume should be sufficient to cover each coverslip around **170 µL** for 8-ibid



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chambers, #80806). For 8-ibid, it recommends incubating the cells at room temperature for **01:00:00** at **Room temperature** if possible.

5 Wash cells with **5 % volume** BSA for **00:05:00** three times. 5m

Incubate cells in secondary antibodies 1h 5m

6 Dilute primary antibody in **5 % volume** BSA and incubate cells at **4 °C** **Overnight** or **01:00:00** at **Room temperature** . 1h 5m

7 Wash cells with **5 % volume** BSA for **00:05:00** three times away from light. 5m

Add Hoechst ( **10 micromolar (µM)** ) in the second wash and leave for **00:15:00** .

8 Take away PBS and load anti-fading medium to cover cells.

For the short term, imaging in PBS is also fine.



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