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Immunofluorescence HeLa cells

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We use this protocol and it's working

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Abstract

Immunofluorescence HeLa cells with BSA/saponin



Day 0

- 1 Plate $1,8 \times 10^5$ HeLa cells on 12 mm coverslips.

Day 1

3h

- 2 Transfect with indicated transfection reagent in DMEM media containing FBS and P/S.

- 2.1 Proceed to next step if not transfecting cells.

- 3 Fix cells adding 8% paraformaldehyde directly into culture media in 1:1 ratio to obtain 4% final concentration of paraformaldehyde.

30m



- 4 Block and permeabilize cells in PBS/0.1% Saponin/3% BSA for at least 00:15:00 at

15m

Room temperature

- 5 Wash coverslips 3X for 00:05:00 with PBS at Room temperature

5m



- 6 Apply primary antibody diluted in PBS/0.1% Saponin/3% BSA and incubate 01:00:00 at Room temperature or Overnight at 4 °C .

1h

- 7 Wash 3X for 00:05:00 with PBS/0.1% Saponin/3% BSA.

5m

- 8 Apply Alexa Fluor secondary antibodies (1:600 from 1 mg/ml stock) diluted in PBS/0.1% Saponin/3% BSA for 01:00:00 at Room temperature in the dark.

1h

- 9 Wash coverslips 3X for 00:05:00 at with PBS/0.1% Saponin/3% BSA.

5m

- 10 Mount coverslips on microscope slides with Prolong Gold mounting medium.



11 Allow to dry o/n at Room temperature in the dark. Store at 4 °C

Day 3

12 Image coverslips at microscope.