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## Immunofluorescent labeling of cultured cells

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**We use this protocol and it's working**

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## Abstract

This protocol describes the process of Paraformaldehyde fixation [PFA-fixation] and immunofluorescent-labeling of cultured cells.

## Troubleshooting

- 1 Wash cells with 1X Phosphate-Buffered solution [1X PBS].
- 2 Fixate cells in 4% PFA for 15 minutes at room temperature [RT].
- 3 Wash cells with 1X PBS twice, for 5 minutes each. Here, cells can be stored in 1X PBS at 4C for up to a week.
- 4 Permeabilize cells with 1x PBS + 0.1% TritonX-100 for 5 minutes at RT.
- 5 Wash with 1X PBS twice, for 5 minutes each wash at RT.
- 6 Block with 10% serum (from species of secondary) for 1 hour at RT.
- 7 Drain and incubate with primary antibody diluted in 5% serum overnight at 4C.
- 8 Wash in 1X PBS thrice, 5 minutes each wash.
- 9 Detect primary antibody with Alexa Fluor secondary antibody diluted 1:400 in 5% serum at RT for 1 hour in the dark.
- 10 Wash in 1X PBS thrice, for 5 minutes each wash at RT.
- 11 Add mounting media with DAPI and apply the coverslip.
- 12 Store slides in the dark at 4C.