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Immunocytochemistry

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We use this protocol and it's working

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Abstract

This protocol describes how to perform immunocytochemistry on 2D fixed cells

Fixing

1 The cells were washed three times with DPBS (GIBCO) and fixed for 10 minutes with 4% paraformaldehyde (Merck Millipore), followed by three more rinses with DPBS.

Blocking and permeabilisation

1h

2 The cells were blocked for 01:00:00 in blocking solution (KPBS 0.25% Triton X-100 (Fisher Scientific) and 5% normal donkey serum) at Room temperature . 1h

Staining

2h

3 The primary antibody was added to the blocking solution and incubated Overnight at Room temperature (follow manufacturer's instructions for dilutions). 1h

4 The cells were then washed the next day three times with KPBS 0.25%.

5 The secondary antibody was added with DAPI (1:1000; Sigma-Aldrich) to the blocking solution and incubated at Room temperature for 01:00:00 . 1h

6 The cells were finally rinsed 2-3 times with KPBS and visualised on a Leica microscope (model DMI6000B).