Basic protocol for Immuno-cytochemistry

1) Fix cells in 4% PFA in 1xPBS 10 min at room temperature.

2) Wash cells with 1xPBS. At this step the cells can be stored in 1xPBS@4 C. For first time test of new antibodies, it is recommended to proceed with staining immediately.

3) Block in 1xPBS, 0,1% Triton X-100, 10 % NGS for 15 min. at room temperature.

4) Add Primary Ab diluted in blocking buffer. Incubate at least 2 hours at room temperature.

5) Wash in 1x PBS for 3 x 5 min.

6) Add secondary ab diluted in blocking buffer incubate 1-2 hours at room temperature.

7) Wash in 1xPBS for 3 x 5 min (add Hoechst to last wash)

8) Mount with Fluoromount G or similar mounting media.

Reagent use: 200 µl pr 18 mm coverslip.

Wash Buffer 1 x PBS

Blocking buffer 1xPBS, 10 % normal goat serum (NGS), 0.1 % triton-x-100. To be made fresh before each staining.

Primary and secondary antibodies are diluted in blocking buffer.

Recommended dilution of secondary antibodies labelled with Alexa flour is 1:1000.
Hoechst can be used at final concentration of 1µg/mL.