

Protocol for Immunostaining of cells

Reagents:

- media without serum
- PBS++ (containing 0.9 mM CaCl₂, 0.52 mM MgCl₂ and 0.16 mM MgSO₄)
- PBS-BSA = PBS++ containing 1% BSA
- 0.2 % Triton-X-100 in PBS++
- 4% PFA (paraformaldehyde) in PBS++
- mounting solution
- cells growing on a coverslip in a 6-well

Procedure:

- wash cells 1x with media without serum and 2x PBS++
- block the cells 15' in PBS-BSA
- wash 2x with PBS++
- fix the cells 20' in 4% PFA
- wash 3x with PBS++
- permeabilize the cells 10' with 0.2 % Triton-X-100
- wash 3x with PBS++
- block the cells 15' in PBS-BSA
- wash 2x with PBS++
- incubate 1 hour with 100 ul primary antibody dilution in PBS-BSA
- wash 3x with PBS++
- block the cells 15' in PBS-BSA
- wash 2x with PBS++
- incubate 30' with 100 ul secondary antibody dilution (containing a fluorescent label) in PBS-BSA (keep cells dark)
- wash 3x with PBS++
- dip coverslip in demi water for a few seconds
- mount cells up site down in mounting solution, let dry for 30' in the dark