# Immunofluorescence: General Cell Protocol

**Please refer to the antibody Product Information Sheet for the primary antibody concentration and the selected cell line.**

1. A multiwell plate (Glass bottom, 96-well, 300µL) is coated with fibronectin (conc. 12.5µg/mL) for 1 hour at room temperature (RT).
2. Cells are seeded (10,000-15,000 cells per well) and incubated at 37°C in humidified air with 5.0% $CO_2$ for at least 4 hours.
3. Growth medium is removed and the cells are washed in PBS (8.1mM $Na_2HPO_4$, 1.5mM $KH_2PO_4$, 137mM NaCl, 2.7mM KCl, pH 7.2).
4. The cells are fixed for 15 minutes in ice cold 4% paraformaldehyde (pH 7.2-7.3) in growth medium supplemented with 10% fetal bovine serum (FBS).
5. The cells are permeabilized 3 times for 5 minutes each with 0.1% Triton X-100 in PBS.
6. The cells are washed once with PBS.
7. The primary antibody is diluted in PBS supplemented with 4% FBS and incubated overnight at 4°C.
8. The following day, the cells are washed 4 times for 10 minutes each with PBS.
9. The secondary antibody is diluted to 1µg/mL in PBS supplemented with 4% FBS and incubated for 1.5 hours at room temperature.
10. The cells are counterstained for 4 minutes with the nuclear probe DAPI.
11. The cells are washed 4 times for 10 minutes each with PBS before mounted in PBS containing 78% glycerol.