

Dual Color Cell Labeling for Polycystin-1 & p58 Protein Protocol

Reagents

Nunc Lab Tek™ Chamber slide Cat.# 178565	0.1% BSA/PBS
1 ⁰ antibody: Mouse anti-Mouse p58K (1:100)	100% Ethanol
1 ⁰ antibody: Rabbit anti-Mouse Polycystin-1 (1:50)	Acetic Acid
2 ⁰ antibody: Donkey anti-Rabbit IgG-FITC (1:100 DAR-FITC)	ddH ₂ O
2 ⁰ antibody: Mouse anti-Mouse IgG-Cy5 or Cy3 (1:100 MAM-Cy5)	PBS

Fixative*

5mls	Acetic Acid (glacial)
25mls	100% Ethanol
<u>20mls</u>	ddH ₂ O
50mls	

Procedure

1. Seed 5X10⁶ cells on Lab-Tek™ chamber one day prior to labeling.
2. Wash cells in PBS (2X with excess volume).
3. Fix cells in Fixative for 30' at 4⁰C.
4. Wash cells in PBS 4X for 5 minutes each at room temp (RT).
5. Wash cells in PBS 1X for 20 minutes each at RT.
6. Incubate cells in both primary (1⁰) antibodies with 0.1% BSA/PBS for 60' in a wet chamber at 37⁰C. Wash cells in PBS 3X for 5' each at RT.
7. Incubate cells in secondary (2⁰) antibody DAR-FITC with 0.1% BSA/PBS 30' at RT. Wash cells in PBS 3X for 5' each at RT.
8. Incubate cells in secondary (2⁰) antibody MAM-Cy5 with 0.1% BSA/PBS 30' at RT. Wash cells in PBS 3X for 5' each at RT.
9. Wash cells in PBS 1X for 10' at RT.
10. Leave a small amount of PBS to cover the cells and analyze by confocal microscopy.

* Note: This fixative is specific for this assay and the cells employed. Optimization of the reagent concentrations was required. Typically cells are fixed in 50% Methanol/ddH₂O.