7TM Antibodies
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7TM Immunocytochemistry Protocol

1. Buffers and Reagents

Use double distilled water for buffer preparation or water with the same grade of purity.

- Blocking buffer: PBS with 3% NGS (normal goat serum)
- Zamboni's Fixative: Preparation of 2 L: Add 80 g Paraformaldehyde to 350 ml saturated picric acid, warm mixture to 60 °C, add 2.52% NaOH drop-wise till solution is clear, filter solution into a 2-L bottle, add phosphate buffer up to 2 L.
- Poly-L-lysine: 0.1 mg/ml
- Phosphate buffer: NaH_2PO_4: 26.5 mM, Na_2HPO_4: 113.4 mM
- PBS: Dulbecco's Phosphate Buffered Saline (NaCl: 137 mM, Na_2HPO_4: 8.1mM, KH_2PO_4: 1.47 mM, KCl: 2.68 mM, pH 7.4)

2. Cell Preparation, Fixation and Permeabilization

1. Place ethanol-sterilized 13-mm coverslips into a 24-well plate and coat with poly-L-lysine for 30 min at room temperature. Aspirate poly-L-lysine. Wash 3-times with water. Aspirate water after each step. Dry plate for 30 min at room temperature.
2. Seed cells into the 24-well-plate onto coverslips and let them grow to a confluence <80%.
3. Treat cells for desired time with or without agonists in fresh media. **Note: To avoid detachment of cells use the rim of the wells to add and remove liquids!**
4. Aspirate media. Wash wells with PBS for 5 min with gentle agitation. Aspirate PBS. Repeat 3-times.

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5. Apply 500 µl Zanboni's fixative and incubate for 30 min at room temperature.
6. Aspirate fixative. Wash wells with PBS for 5 min with gentle agitation. Aspirate PBS. Repeat 3-times.
7. Permeabilization: Apply 500 µl 50% ice-cold methanol for 3 min. Aspirate methanol. Apply 500 µl 100% ice-cold methanol for 3 min. Aspirate methanol.
8. Wash wells with PBS for 5 min with gentle agitation. Aspirate PBS. Repeat 3-times.

3. Blocking, Antibody Incubation and Mounting

1. Incubate wells for 1 hour in blocking buffer at room temperature with gentle agitation.
2. Incubate wells with 500 µl of Premium 7TM Antibodies at a dilution of 1:200 in PBS with 1% NGS for 1-2 hours at room temperature or at 4°C overnight with gentle agitation.
3. Wash wells with PBS for 5 min with gentle agitation. Aspirate PBS. Repeat 3-times.
4. Incubate wells with anti-rabbit fluorochrome-coupled secondary antibody of your choice in PBS with 1% NGS for 1-2 hours at room temperature or at 4 °C overnight with gentle agitation **in the dark**.
5. Wash wells with PBS for 5 min with gentle agitation **in the dark**. Aspirate PBS. Repeat 3-times.
6. Mount coverslips by using a suitable mounting media on microscope slides. Store at 4 °C **in the dark**.