

7TM Surface ELISA Protocol

Note: This protocol is only suitable for detection of receptor internalization of N-terminal HA- or FLAG-tagged GPCRs. This procedure is also known as "antibody feeding".

1. Buffers and Reagents

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

- Blocking buffer: PBS with 10% 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: 0.2 mg/ml
- PBS: Dulbecco's Phosphate Buffered Saline (NaCl: 137 mM, Na₂HPO₄: 8.1mM, KH₂PO₄: 1.47 mM, KCl: 2.68 mM, pH 7.4)

2. Cell Preparation, Primary Antibody Incubation and Fixation

- Coat 24-well plate 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 60 min at room temperature.
- 2. Seed cells into the 24-well plate and let them grow to a confluence <80%.
- 3. Aspirate media. Note: To avoid detachment of cells use the rim of the wells to add and remove liquids!
- Incubate wells with 150 μl of Premium 7TM Epitope Tag Antibodies at a dilution of 1:500 in serum-free media in a refrigerator for 1-2 hours at 4 °C. Aspirate antibody solution.



- 5. Wash wells with ice-cold serum-free media. Aspirate media.
- Treat cells for desired time with or without agonist in fresh media at 37 °C in a CO₂ incubator.
- 7. Aspirate media. Wash wells with ice-cold PBS. Aspirate PBS.
- 8. Apply 500 µl Zamboni's fixative and incubate for 30 min at room temperature.
- Aspirate fixative. Wash wells with PBS for 5 min with gentle agitation. Aspirate PBS. Repeat 3-times.

3. Blocking, Secondary Antibody Incubation and Detection

- 1. Incubate wells for 1 hour in blocking buffer at room temperature with gentle agitation.
- Incubate wells with anti-rabbit HRP-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.
- Wash wells with PBS for 5 min with gentle agitation in the dark. Aspirate PBS. Repeat 3-times.
- Apply 250 µl of peroxidase substrate of your choice (prewarmed to room temperature!) to each well.
- 5. Stop peroxidase reaction by transferring 200 μ l to a 96-well plate.
- 6. Use a plate reader to determine receptor internalization.