

ELISA: Sandwich ELISA Protocol - Membrane Proteins

Materials

- Maxisorb 96-well plate
- Goat anti-mouse IgG, unconjugated
- Goat anti-rabbit IgG, peroxidase conjugated
- Specific monoclonal mouse antibody as capture antibody
- Specific polyclonal rabbit antibody as detector antibody
- Protease inhibitors (suggested: 1 mM PMSF, 1 µg/ml Aprotinin, 1.5 µM Pepstatin A)
- Microplate absorbance reader with filters at 450 nm and a reference wavelength (e.g. 620-650 nm)

Reagents

- Coating buffer: 0.1 M Na-carbonate, pH 9.6 (store 0.5 M stock at -20°C)
- Blocking buffer A: 1% tryptone/peptone from casein (TP) in 0.1 M Na-carbonate, pH 9.6
- Washing buffer: Tris buffered saline (TBS) with 0.05% Tween 20 (TBST)
- Solubilization buffer A: 10% sodium dodecyl sulfate (SDS) in PBS
- Solubilization buffer B: 1.2% Triton X-100 in PBS
- Antigen buffer A: 0.2% Triton X-100/0.05% TP in TBS
- Antigen buffer B: 0.05% TP in TBS
- Blocking buffer B: 0.5% TP/0.5% BSA/0.5% gelatin in TBST
- Substrate solution: Tetramethylbenzidine (TMB) reagent for development
- Stop solution: 0.25 M H₂SO₄ to stop development

Procedure

- 1. Coat 96-well microplate with 100 µl goat anti-mouse IgG (1 µg/ml) in coating buffer and incubate for 3 h at RT and 700 rpm.
- 2. Block the surface with **blocking buffer A** for 1 h at RT and 700 rpm.
- 3. Wash the plate three times with washing buffer (at least 5 min per wash) and transfer them to 4°C.
- 4. Apply monoclonal capture antibody diluted in washing buffer and incubate overnight at 4°C. Dilute ascites 1:4000, purified antibody 1:2000 (50-75 ng/well).
- 5. Antigen solubilization: Adjust protein standard and samples to 3 mg/ml total protein and a final SDS concentration of 1.2% with solubilization buffer A and rotate 15 min at RT. Add 5 volumes of ice-cold solubilization buffer B to each sample and rotate 15 min at 4°C. Pellet the insoluble fraction at 100,000x g for 30 min (acceptable alternative: 13,000 rpm for 30 min at 4°C in a tabletop centrifuge) and transfer the supernatant to a new tube. Dilute the supernatant in antigen buffer B to 0.2% Triton X-100 concentration. Note: If complete tissue samples are used, DNase should be added as 0.1 µg/µl together with protease inhibitors, and SDS should be added as last component after mixing everything else.
- 6. Wash the plate once with washing buffer, twice with antigen buffer A at RT.
- 7. Apply antigen diluted in antigen buffer A and incubate for 2 h at RT and 700 rpm.
- 8. Wash twice with antigen buffer A, once with blocking buffer B.
- 9. Incubate with **blocking buffer B** for 30 min at RT.
- 10. Apply polyclonal detector antibody diluted in blocking buffer B (dilution 1:1000 up to 1:8000) and incubate for 1 h at RT and 700 rpm.
- 11. Wash three times with **blocking buffer B**.
- 12. Incubate with HRP-coupled goat anti-rabbit antibody, diluted 1:10,000 in blocking buffer B, for 1 h at RT and 700 rpm.
- 13. Wash three times with washing buffer.
- 14. Add 100 µl substrate solution for development.
- 15. Stop the reaction after 30 min with 100 µl stop solution and read the absorbance at 450 nm (ref: 620-650 nm).

Reference

Geumann C, Grønborg M, Hellwig M, Martens H & Jahn R (2010). A sandwich enzyme-linked immunosorbent assay for the quantification of insoluble membrane and scaffold proteins. Analytical Biochemistry 402: 161-9.