Protocol for GAD2 Antibody (Cat. No. 198 103)
Sandwich Enzyme-linked Immunosorbent Assay (ELISA)
Transmembrane Proteins

The Enzyme-Linked Immunosorbent Assay (ELISA) is a highly sensitive and specific method used to detect and quantify various biomolecules, including proteins, peptides, antibodies, and hormones. In a **sandwich ELISA**, a capture antibody is first immobilized on a solid surface, typically the wells of a microtiter plate. This antibody selectively binds to the target molecule present in the sample (analyte). Subsequently, a detector antibody—specific to a different epitope on the same target—binds to the captured molecule. Detection is achieved through an enzymatic reaction facilitated by a secondary antibody that is conjugated to an enzyme, producing a measurable signal.

Materials

- **ELISA-Plate**: Maxisorb 96-well plate
- **Microplate shaker**
- **Microplate absorbance reader:** Equipped with filters at 450 nm and a reference wavelength (e.g. 620-650 nm)
- Protease inhibitors (suggested: 1 mM PMSF, 1 µg/ml Aprotinin, 1.5 µM Pepstatin A)

Reagents

- **Capture antibody A:** Unconjugated carrier free goat anti-rabbit IgG
- **Capture antibody B:** rabbit anti-GAD2 antibody (cat. no. 198 103)
- **Detector antibody**: mouse anti-GAD2 antibody (cat. no. 198 111)
- **Secondary detection reagent:** Horseradish peroxidase (HRP) conjugated anti mouse secondary antibody
- **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 coating buffer
- **TBS:** Tris buffered saline (50 mM Tris, 150 mM NaCl), pH 7,2
- **TBST:** Tris buffered saline with Tween 20 (50 mM Tris, 150 mM NaCl, 0.05% Tween 20), pH 7,2
- **PBS:** Phosphate buffered saline, (200 mM NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4), pH 7.4
- **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 H_2SO_4 to stop development

Procedure

1. Coat 96-well microplate with 100 µl **Capture antibody A** (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 **TBST** (at least 5 min per wash) and transfer them to 4°C.

4. Apply **capture antibody B** diluted in **TBST** and incubate overnight at 4°C at a **dilution of 1:2000**.
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.
6. Add 5 volumes of ice-cold **solubilization buffer B** to each sample and rotate 15 min at 4°C.
7. 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.
8. 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.
9. Wash the plate once with **TBST**, twice with **antigen buffer A** at RT.
10. Apply antigen diluted in **antigen buffer A** and incubate for 2 h at RT and 700 rpm on a **microplate shaker**.
11. Wash twice with **antigen buffer A**, once with **blocking buffer B**.
12. Incubate with **blocking buffer B** for 30 min at RT.
13. Apply **detector antibody** diluted in **blocking buffer B (dilution 1:1000 up to 1:8000)** and incubate for 1 h at RT and 700 rpm on a **microplate shaker**.
14. Wash three times with **blocking buffer B**.
15. Incubate with **secondary detection reagent** (diluted according to the manufacturer's recommendations) in **blocking buffer B**, for 1 h at RT and 700 rpm on a **microplate shaker**.
16. Wash three times with **TBST**.
17. Add 100 µl **substrate solution** for development.
18. 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.