Protocol for Oligo-Abeta-pE3 Antibody (Cat. No. 218 511)
Native Western Blot (WB)
ECL Detection

In native Western blot (WB) approaches, protein conformation and complexes stay intact. Their migration properties depend on size and individual charge. After transfer to a membrane proteins or protein complexes can be analyzed by immuno-detection with antibodies. Enhanced chemiluminescent (ECL) detection systems and substrates have different sensitivities and have a narrow linear detection range that can be used for protein quantification. In general, the experiment has to be carefully optimized for reliable results.

Materials and reagents

- **Methanol (100%)**
- **TBST - skimmed milk:** (20 mM Tris, 150 mM NaCl, 5% (w/v) skimmed milk powder, 0.1% Tween 20), pH 7.4
- **Washing solution A:** (20 mM Tris, 150 mM NaCl, 0.1% Tween 20), pH 7.4
- **Washing solution B:** (20 mM Tris, 150 mM NaCl), pH 7.4
- **Primary antibody:** Oligo-Abeta-pE3 antibody (cat. no. 218 511)
- **Secondary antibody:** Horseradish conjugated mouse specific secondary antibody.
- **Substrate solution:** Western Lightning® Plus-ECL PerkinElmer, Inc. or comparable product

Procedure

Important: To keep protein complexes or conformation intact run PAGE with non-boiled samples. We recommend the NativePAGE system from invitrogen.

1. Monomerize synthetic Abeta peptides in 70 % formic acid, and evaporated solvent in a speed-vac immediately. Store dried peptides at -80°C.
2. Prior to each experiment, dissolve peptide in 0.3 % ammonia and perform ultrasonic treatment, and dilute to an end concentration of 0.15 % ammonia.
3. Prepare samples (2,5 µg / lane for a 10 lane mini-gel) in NativePAGE sample buffer according to the manufacturer's instructions.
4. Separate the protein sample to be examined and a NativePAGE compatible molecular weight standard by NativePAGE. Follow the manufacturer's instructions for your PAGE device.
5. Activate the PVDF membrane by incubating it for several minutes in 100% methanol. Remove excess of alcohol with clean paper towel.
6. Transfer Protein to PVDF membrane by electro-blotting. Follow the manufacturer's instructions for your blotting device.
7. Briefly rinse the membrane with destilled water.

8. Boil membrane for 3 min in destilled water and let cool down at room temperatur (RT).
9. Incubate in **TBST - skimmed milk** for 30 min on an orbital shaker at RT.
10. Incubate in fresh **TBST - skimmed milk** containing the **primary antibody** at a dilution of **1:500** for at least 2 h on an orbital shaker at RT or overnight at 4°C.
11. Wash 3-4 times with **washing solution A** for 10 min each time.
12. Incubate with fresh **TBST - skimmed milk** containing the **secondary antibody** diluted according to the manufacturers instructions for at least 1 h on an orbital shaker.
13. Wash 3 times with **washing solution A** for 10 min each time.
14. Replace **washing solution A** with **washing solution B** and let equilibrate for 5 min.
15. Replace with fresh **substrate solution** and develop (X-ray film or ECL-reader). Exposure time can be shortened or extended, if signals are extremely strong or weak, resp.

Remarks

A very weak signal may be caused by the primary and/or secondary antibody concentration being too high. The ECL substrate solution has a limited capacity, and high amounts of local peroxidase can use up all the substrate within seconds before the picture is taken in your ECL reader.
Please try a lower concentration of primary and secondary antibodies in this case.

Note: This protocol has been validated in the SYSY Antibodies laboratories to ensure consistent and reliable staining results. However, for achieving the best specific signal with minimal background, the optimal antibody concentration, incubation temperature, and incubation duration should be optimized for each experiment.