A□ ELISA Xiaoyan Sun (04052004)

The sandwich A□ ELISA protocol was originally developed in Dr.Cole' lab of UCLA and Dr.Takashima's Lab of RIKEN, Japan. It is to quantify A□x-40 and A□x-42 by using the end-specific monoclonal antibodies as the capture antibodies respectively. A biotinylated monoclonal anti-N terminus A□ secondary antibody is used as the detection antibodies. Streptavidin-conjugated alkaline phosphatase and AttoPhos (Amersham) are used as the reporter system. Attophos fluorescence is obtained with 444 nm excitation and emission at 555 nm.

1. NUNC Maxisorb immunoassay plates are coated with 0.3 □g/well capturer antibodies in PBS overnight at 4□C (48 hours for low concentration of antibodies).
2. Plates are subsequently blocked with Block ACE (Japan,1:4 dilution of original solution) for 2 hours at room temperature. Wash plate with PBS-T briefly. Load the samples in the wells.
3. Incubate samples with antibodies overnight at 4□C. Wash with PBS-T twice.
4. The plates are then incubated in a solution of the detector antibody for 2 hours at 4°C.
5. Wash the plate with PBS-T twice followed by treating the plates with the alkaline phosphatase for 1.5 hour (Streptavidin-conjugated alkaline phosphatase, Amersham, 1:5000) at 4°C.
6. Wash plates with TBS-T twice. The signal is amplified by adding 100 □l AttoPhos and measures with a Fluoroskan (Labsystems, Finland).

Construction of a standard curve

For construction of standard curves A□1-40 and A□1-42 peptides (10ug/ml) (Anac, USA) are dissolved in Block Ace (1:10 dilution of original solution with 0.05% Tween 20) or 3% BSA with 0.05% tween. Serial dilution of amyloid peptides is performed (sensitivity, 3.125pg/ml).

Reference

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2. Lithium inhibits amyloid secretion in COS7 cells transfected with amyloid precursor protein C100 Neuroscience Letters 321 (2002) 61–64
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