96 well Abeta ELISA using Fluorescent substrate
Using white Nunc MaxiSorp 96 well plates (Fisher cat. # 12-565-290A-nunc#437796)

Day 1:
1. Coat with 100ul/well of coating antibody (82E1 0.5 ug/ml) diluted in filtered PBS (sigma cat # P-3813). 10 mls needed for one 96 well plate.
Incubate plate O/N at 4°C covered with plate sealer.

Day 2:
2. Block plates with 200ul/well of 1% Block ACE powder in H2O (Dainippon Pharmaceutical Co Japan cat # UK-B80 $165.00/box) for 3hrs at RT covered with plate sealer.

a. Dissolve 4g BlockACE powder in 100ml deionized water. Dilute a portion of concentrated BlockACE 1:4 in deionized water to final 1% for blocking.

b. Dilute the other portion of concentrated BlockACE 1:10 (to final 0.4%) in sterile-filtered PBS-T (sigma cat # P-3563) for diluting standard.

3. Wash plates: PBS-T (0.05% tween-20) 250ul/well; 3x 30 seconds—use plate washer

4. Load 100ul of standards or samples ** freshly diluted in 0.4% BlockAce in PBS-T for 2 hr at RT.

Day 3:
5. Incubate 100ul/well of reporter antibody (82E1B 12 ng/ml) diluted in PBS for 2 hours at RT covered with plate sealer; 10 mls needed for 1 plate.

6. Incubate 100ul/well of streptavidin alkaline phosphatase (Promega cat # V5591 $77.00/0.5ml) 1:5000 dilution in PBS for 1 hour at RT covered with plate sealer.

7. Wash plates: TBS 250ul/well; 3x 30 seconds—use plate washer

8. Amplify signal by adding 100ul/well AttoPhos Fluorescent substrate system (Promega cat # S1000 $230.00) for 3 min at RT. 100mg of AttoPhos substrate should be mixed with 166ml of AttoPhos buffer 24 hours prior to use.

9. Signal measured on Fluorometer (Victor2 Perkin Elmer); excitation: 440nm; emission: 550nm