Instructions for Generating a Calibration Curve with Pre-Formed Oligomeric Aβ Peptides

Background Information
This application note provides detailed instructions for generating a calibration curve with PE-1750-1000, for use in the Biosensis Oligomeric Aβ ELISA Kit (BEK-2215). It substitutes instructions given in Section 7 of the BEK-2215 kit manual. Note that PE-1750-1000 is supplied as 2 x 500 ng vials.

Preparation of Standard Curve

1. Equilibrate one 500 ng vial to room temperature; the lyophilized material may appear wet, which is due to the hygroscopic nature of the stabilizing buffer components and does not affect product quality
2. Spin the vial briefly to collect the material at the bottom of the tube
3. Add 1 mL of Assay Diluent B to the vial and let stand for 10 minutes at room temperature
4. Vortex and bring the lyophilized material into solution. Inspect the vial to confirm that no pellet material is left at the bottom of the tube
5. The concentration of oligomeric Aβ is now 500 ng/mL, label the tube accordingly.
6. Prepare a standard curve within 2.0 – 0.03 ng/mL, with 1:2 serial dilution. The volumes used for the dilution series depends on the number of repeats per oligomeric Aβ peptide standard. For triplicate measurement (100 µL per well) of each oligomeric Aβ standard (o-Aβ) concentration, you may want to follow this procedure:
   I. Label 7 tubes with 2 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.25 ng/mL, 0.125 ng/mL, 0.0625 ng/mL and 0.0313 ng/mL, respectively
   II. Aliquot 400 µL of the assay diluent into each tube except the tube labeled “2 ng/mL”
   III. Dilute the 500 ng/mL o-Aβ stock solution 250-fold (4 µL of o-Aβ stock solution added to 996 µL assay diluent); the concentration of o-Aβ is 2 ng/mL
   IV. Take 400 µL from the “2 ng/mL” tube and transfer to the tube labeled as “1 ng/mL”
   V. Repeat step IV. for each consecutive concentration until the last tube “0.0313 ng/mL” is prepared

Note: Pipet up- and down and vortex to mix; to avoid foaming, use only a very brief vortex.

7. The calibration curve standards are now ready to be loaded into the microplate wells and should be used within 1 hour; refer to the BEK-2215 kit insert for the assay procedure and instructions on calculating results.
8. The reconstituted 500 ng/mL stock solution may be stored at 2-8° for up to 2 days, and should not be frozen for best results.

IMPORTANT: While the concentration of monomeric Aβ peptide used to form the oligomeric complexes is accurately determined, the precise formation, size and number of oligomers cannot be quantified by any known method. We recommend results should be presented as "Aβ peptide equivalents; ng/mL (Arbitrary Units)".
Typical Data

Oligomeric Aβ peptide calibration curves were generated with the HFIP-treated Aβ peptide supplied in the Oligomeric Aβ ELISA kit (BEK-2215), also available as PE-1749-50, and compared against pre-formed, lyophilized oligomeric Aβ peptides (PE-1750-1000).

This graph demonstrates the stability of pre-formed oligomeric Aβ peptides and the usefulness of PE-1750-1000 as calibration curve standard for BEK-2215 and other oligomeric Aβ ELISA kits.