Instructions for Reconstituting the HFIP-Treated Peptide

This instruction sheet provides a detailed procedure for reconstituting the supplied HFIP-treated Aβ peptide to ensure that the material is fully dissolved and provides optimal conditions for further use as unaggregated, oligomerized, fibrillar or complexed peptide.

1. Hold the supplied vial of HFIP-treated Aβ peptide against bright light and inspect for the presence of either a translucent film of lyophilized peptide at the bottom of the tube, or translucent, small “droplets” distributed over the inside wall of the vial (marked with red arrow below).

Lyophilized Aβ peptide

2. Add 5 µL of reconstituting buffer and spin briefly to collect the liquid at the bottom of the tube. Vortex at highest speed for 5 seconds, rotating the vial with your hands; the liquid should distribute inside the tube and cover a large area. To dissolve all peptide material, harsh vortexing is required. Spin down the liquid and repeat the vortex-spin procedure for a total of 3 times.

   Inspect the vial as in step 1. If “droplets” (marked with red arrow below) are still visible after the last centrifugation step, the reconstitution procedure (vortex-spin) has to be continued, because the peptide is not yet fully dissolved.

Aβ peptide not fully dissolved

   Important: Do not add more reconstituting buffer to the vial to facilitate dissolving the lyophilized peptide. A higher volume of reconstituting buffer will affect the ability of Aβ to oligomerize, form fibrils or complexes with other proteins.

3. Continue to vortex the vial and collect the liquid at the bottom of the tube. Inspect the vial as in step 1. Once all “droplets” have disappeared and are collected at the bottom of the tube after centrifugation, the HFIP-treated peptide is fully dissolved (see photo below).

Aβ peptide fully dissolved

   Note that the procedure may require several minutes to fully reconstitute the peptide.

4. Add 106 µL of diluent (depending on application) to make up to 111 µL total volume.

5. Repeat the vortex-spin procedure 3 times as before.

6. Final peptide concentration is 100 µM (450 µg/mL).

7. Use immediately for unaggregated Aβ or Aβ complexes, incubate at 2-8°C for 24 hours (protected from light) for oligomerized Aβ, or incubate at 37°C for fibrillar Aβ peptide.