Protocol: Extraction of human Aβ from APP Tg mouse brains into TBS-soluble, guanidine HCl-soluble fractions

TBS-soluble fraction

1) Make 10 ml of fresh TBS extraction buffer (sterile 1x TBS + 100 µl of 200 mM 1,10 phenanthroline + 100 µl of 500 mM EDTA + 1 tab of Roche complete EDTA-free Mini protease inhibitor).

TBS extraction buffer final concentrations:

- 140 mM NaCl
- 3 mM KCl
- 25 mM Tris-HCl, pH 7.4
- 1 tab/10 ml of Roche complete EDTA-free Mini protease inhibitor
- 5 mM EDTA
- 2 mM 1,10-phenanthroline

2) Weigh frozen hemisphere quickly, before it thaws. After weighing, keep brain in a weigh boat on dry ice until homogenization.

3) Put 4 brain volumes (4 µl/mg of brain) of ice-cold TBS extraction buffer into a 2 ml Potter-Elvehjem (PE) homogenizer on ice, then add the brain hemisphere.

4) Attach the pestle to a motorized rotor and homogenize sample. Do 20 strokes.

5) Transfer homogenate (with a long-necked transfer pipette) to an ultracentrifuge tube, and store on ice until all samples are homogenized.

6) Weigh tubes and arrange them into weight-matched pairs. Put each member of the pair across from its match in the rotor.

7) Spin at 100,000xg for 1 hr at 4ºC. Save the supernatant as the "TBS-soluble fraction".

Guanidine HCl-soluble fraction

1) Add 4 volumes (based on initial hemisphere weight) of cold 6.25 M guanidine HCl in 50 mM Tris, pH 8.0 to the pellet.

2) Vortex the tube until the pellet becomes dislodged from the bottom.

4) Transfer sample to a clean PE homogenizer. Make sure all pellet fragments are transferred and not stuck in the tube or pipette.

5) Using the motorized pestle to homogenize sample with 20 strokes.

6) Transfer the homogenate with a long-necked transfer pipette to a new tube.

7) Nutate for ~2 hr at RT, then spin at 4ºC 20,800xg for 20 minutes. Save supernate as the "GuHCl-soluble fraction," and discard the small viscous pellet.