Preparation of Brain Samples for LEGEND MAX™ Beta Amyloid ELISA

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[1]BioLegend

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Working

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PROTOCOL STATUS

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GUIDELINES

Notes on Preparation and Assaying of Samples

[1]EDTA in homogenization buffer helps solubilize Aβ and increases recovery of Aβ in the soluble fraction by as much as 50% for mild β-amyloid loaded brain (Cherny, 1999, JBC). HOWEVER, it is unclear if EDTA-solubilized material is free in solution or a precipitate in vivo. Mice at different ages/stages give different soluble/chelator yields (presumably because of shifts in quantity/quality of chelator yields (presumably because of the shifts in levels in S1/EDTA fractions ("soluble" Aβ) from different aged mice.

[2]Choice of type of detergent or formic acid will depend on experimental circumstance:
Non-ionic Triton X-100 extracts a portion of insoluble Aβ. As with chelators, the portion of insoluble Aβ extracted by non-deterents is dependent on age/stage of mice. For early stage of β-amyloid deposition (i.e. mice >12 months) up to 80% of total mouse brain Aβ is extracted by Triton X-100. Proportion drops rapidly 20% or less in old animals (i.e. mice > 18 months) with heavy mature β-amyloid deposition. Biolegend's Aβ ELISA's (and most other ELISA's) are compatible with non-ionic detergents.

The ionic detergent SDS gives more complete Aβ extraction from insoluble pellets (> 90% with young and ≈ 50% for older mice). HOWEVER, SDS is not compatible with many ELISA systems. Biolegend Aβ kits are resistant to SDS and samples with ≤ 0.2% SDS can be used with minimal loss in sensitivity (< 10%). Hence, E1 fractions with SDS should have a final in assay dilution of at least 3-fold.

Early protocols used formic acid to extract Aβ from AD brain. For mature β-amyloid deposits in human and old mice brain the highest extraction yields are still observed with this buffer. HOWEVER, a difficult multi-step process is required to prepare formic acid fractions for assay (both E1 and whole tissue extracts). Normalization between samples is also problematic.

Steps to prepare formic acid extracts for Aβ assay

The bulk of the formic acid buffer needs to be evaporated off (down to 10-20% of original vol) with a nitrogen stream. If dried completely formic acid brain samples form a plastic-like pellet that is highly resistant to resolubilization.

Aliquots of the remaining material must be neutralize (to pH 7) with 5 M NaOH in 1M Tris. Indicator should be added to the neutralization buffer to signal when pH 7 is reached.

Assay immediately - DO NOT FREEZE neutralized samples (precipitates of highly enriched insoluble hydrophobic species will form that interfere with assay signal).

Care should be taken to have equivalent pH for all samples. Neutralized samples should have a final in assay dilution of at least 10-fold. Normalizing between samples is problematic. Residual formic acid interferes with most protein assays and protein levels may not be proportional to starting materials in any case. Careful attention to maintaining equivalent sample volumes is often the most practical approach.

TBS = Tris, pH 7.4 (50 mM) + NaCl (150 mM)

Soluble and insoluble tissue fractionation - This protocol generates soluble and insoluble subcellular fractions for analysis of Aβ partitioning in tissues

1 Homogenize in TBS with protease inhibitors (Pierce sells excellent solid tablets for use with homogenization buffers) at 5mLs per 1g

tissue. Teflon/glass homogenizer with 5-6 passes on ice. EDTA1 (2mM) can be a useful addition to homogenization buffers (See Guidelines).

2 Spin for 20 minutes 350,000xg (or equivalent). 00:20:00

3 Remove supernatant (S1 or soluble fraction) - usually contains < 5 % of the total brain Aβ pool in young mice decreases with age.

4 Resuspend pellet (agitate to break up) with: (use only one method below)

a. Detergent2 - 1% triton OR 0.6% SDS (see notes) in TBS/inhibitor buffer using same volume as homogenization step (15 minutes incubation).
b. 70% Formic acid2, 40-50% of homogenization volume (incubate 30 minutes).

5 Spin a second time (20 minutes x 350,000 g or equivalent) and remove supernatant - detergent or formic acid extract fraction (E1). 00:20:00

6 **Optional** - if detergent extract was prepared above, then remaining pellet can be further extracted with formic acid - 70% formic acid, half homogenization volume, spin down 10 minutes in microfuge full speed (10 minutes x 14,000 g). Remove formic acid extract (E2). 00:10:00

7 S1 and E1 fractions are best assayed immediately but can be stored at -20°C (including non-neutralized formic acid samples).

Whole tissue extraction - This protocol generates a single fraction containing the total Aβ tissue load

8 Homogenize tissue as above in TBS with protease inhibitors and EDTA.

9 To homogenate add concentrated Triton X-100, SDS (to final 1 or 0.6% respectively) or formic acid (to final 70%). Agitate by pipetting up and down to mix. Detergents and formic acid are added after tissue homogenization to reduce foaming and other potential problems.

10 Spin for 20 minutes x 350,000 g (or equivalent). 00:20:00

11 Remove supernatant for assay (whole brain extract).

NB High salt and biomolecule concentrations in formic acid whole tissue extracts can sometimes result in visible precipitants forming in later steps. These samples retain strong Aβ signal but we have not established to what degree signal attenuated under these conditions.