

# 26th Annual Alzheimer Day

## **Soluble protein extraction from paraformaldehyde (PFA) and neutral buffered formalin (NBF) fixed paraffin embedded human brain tissue**

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**Introduction** Human brain banking is an essential part of examining neurodegenerative proteinopathies, such as Amyloid Beta ( $A\beta$ ), a primary pathologic culprit in Alzheimer's Disease (AD). Studies have demonstrated correlations between cognitive impairment and soluble  $A\beta$  ( $sA\beta$ ) levels. Furthermore,  $sA\beta$  from frozen human tissue has shown threefold higher levels in AD brain samples when compared to healthy controls. However, the majority of human brain banked tissue is fixed and embedded in paraffin blocks. We have optimized and validated an efficient reproducible procedure for extraction of soluble proteins from PFA and NBF fixed paraffin embedded human brain tissue which can then be quantified using assays for multiplexing and increased sensitivity such as Meso Scale Discovery (MSD).

**Methods:** Four brains from the Northwestern University Alzheimer's Disease Center brain bank, with increasing Consortium to Establish a Registry for Alzheimer's disease (CERAD) scores, were fixed with either PFA or NBF, and sections were embedded in paraffin blocks. Blocks of neocortex were then cut into twelve curls, each ten microns thick. Curls were then placed in microtubes totaling 30, 40, and 50 microns and deparaffinized in xylene. The Qiagen QProteome FFPE Tissue Kit was used to extract proteins from the soluble fraction. Samples were analyzed for total protein concentration via BCA. Western blots were performed for validation to confirm presence and amount of each viable protein prior to quantitative assessments using MSD assay.

**Results:** BCA findings revealed 40 microns of fixed tissue produces optimal sample concentrations when using the Qiagen kit. Western blots confirmed extraction of soluble

proteins including; A $\beta$ , glial fibrillary binding protein (GFAP), and human tau-DNA binding protein 43 (hTDP-43).

**Conclusions:** Effective extraction of soluble proteins from PFA and NBF fixed paraffin embedded human tissue provides a useful approach for quantitative comparative studies of neurodegenerative disease associated proteins and other biomarkers.

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