Preparation of NFTs from AD Brain Tissue

Reference

1. Reynolds, M. R., Reyes, J. F., Fu, Y., Bigio, E. H., Guillozet-Bongaarts, A. L., Berry, R. W., & Binder, L. I. (2006). Tau nitration occurs at tyrosine 29 in the fibrillar lesions of Alzheimer's disease and other tauopathies. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(42), 10636–10645. https://doi.org/10.1523/JNEUROSCI.2143-06.2006

Solutions

1. MES Homogenization Buffer:100 mM MES, 0.75 M NaCl, 1 mM EGTA, 0.5 mM MgSO4, pH 6.5
2. PHF/NFT Extraction Buffer:10 mM Tris, 10% Sucrose, 0.85M NaCl, 1 mM EGTA, pH 7.4
3. Tris-HCl: 50 mM Tris, pH 7.4

Abbreviations

1. MES: 2-(N-morpholino)ethanesulfonic acid
2. PHF: Paired Helical Filament
3. NFT: Neurofibrillary Tangles
4. HCl: Hydrochloric Acid

Protocol

1. Homogenize 50-100 mg tissue in 200 µL MES Homogenization Buffer.
2. Centrifuge at 11,000 x g, 4°C for 20 minutes.
3. Discard pellet, centrifuge supernatant at 100,000 x g, 4°C for 60 minutes.
4. Save supernatant, resuspend pellet in 200 µL PHF/NFT Extraction Buffer
5. Centrifuge at 15,000 x g, 4°C for 20 minutes.
   Supernatant = Isolated PHFs / small PHF aggregates
   Pellet = Intact, fragmented NFTs and larger PHF aggregates
6. Save supernatant, re-extract pellet in PHF/NFT Extraction Buffer and centrifuge at 15,000 x g, 4°C for 20 minutes.
7. Pool supernatants and add 1% Sarkosyl and mix by agitation at RT.
8. Centrifuge 100,000 x g, 4°C for 60 minutes.
9. Resuspend pellet in 50 mM Tris-HCl pH 7.4 Buffer using 1 µL buffer for each mg of initial tissue. Label as “NFT Prep”.