Methods for TEM

Below is the standard methods we use for TEM sample prep. Please note this is for reference only and should not be copied and pasted directly into manuscripts.

Cell culture samples on Thermanox plastic or glass coverslips(number 2 thickness required) in a 24 well plate were fixed in 0.1 M sodium cacodylate buffer pH7.35 containing 2% paraformaldehyde and 2.5% glutaraldehyde and post-fixed with 2% osmium tetroxide in unbuffered aqueous solution, rinsed with distilled water, en bloc stained with 3% uranyl acetate, rinsed with distilled water, dehydrated in ascending grades of ethanol, transitioned with 1:1 mixture of ethanol and resin, and embedded in resin mixture of Embed 812 kit, cured in a 60˚C oven. Samples were sectioned on a Leica Ultracut UC6 ultramicrotome. 70 nm thin sections were collected on 200 mesh copper grids, post stained with 3% uranyl acetate and Reynolds lead citrate.

*Please note that the fixative for neurons in not 2% paraformaldehyde and 2.5% glutaraldehyde, instead it is only 2.5% glutaraldehyde. Please contact Lennell to verify what fixative was used.

Tissue samples were fixed in 0.1 M sodium cacodylate buffer pH7.35 containing 2% paraformaldehyde and 2.5% glutaraldehyde and post-fixed with 2% osmium tetroxide in unbuffered aqueous solution, rinsed with distilled water, en bloc stained with 3% uranyl acetate, rinsed with distilled water, dehydrated in ascending grades of ethanol, transitioned with propylene oxide and embedded in resin mixture of Embed 812 kit, cured in a 60˚C oven. Samples were sectioned on a Leica Ultracut UC6 ultramicrotome. 1 um thick sections were collected and stained with Toluidine Blue O and 70nm sections were collected on 200 mesh copper grids; thin sections were stained with uranyl acetate and Reynolds lead citrate.

Cell Pellet samples were fixed 0.1 M sodium cacodylate buffer pH7.35 containing 2% paraformaldehyde and 2.5% glutaraldehyde. Then mixed with 3% agarose and placed back into initial fixative for 1 hour. Rinsed 3 times in distilled water, post-fixed with 2% osmium tetroxide in unbuffered aqueous solution, rinsed with distilled water, en bloc stained with 3% uranyl acetate, rinsed with distilled water, dehydrated in ascending grades of ethanol, transitioned with propylene oxide and embedded in resin mixture of Embed 812 kit, cured in a 60˚C oven. Samples were sectioned on a Leica Ultracut UC6 ultramicrotome. 1 um thick sections were collected and stained with Toluidine Blue O and 70nm sections were collected on 200 mesh copper grids; thin sections were stained with uranyl acetate and Reynolds lead citrate.

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