

## 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 pH7.3 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.

**Tissue samples** were fixed in 0.1 M sodium cacodylate buffer pH7.3 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 pH7.3 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.