Beta amyloid uptake by glial cells

A detailed step-by-step protocol on how to measure beta amyloid uptake by glial cells.

A. Prepare conditioned media and cells on Day 1

1. Grow 293sw cells (using G418 DMEM) to complete confluence, then remove media and replace it with DMEM without any drug, 10 ml each plate.
2. Add 2 ml of stock poly-Lysine solution into 200 ml of autoclaved H_2O and sterilize solution with filter. Cover each petri dish with 5 ml of poly-Lysine for at least 20 min inside the hood. Aspirate poly-Lysine and let it air dry inside the hood.
3. Transfer conditioned media of BV2 cells to a fresh 50 ml conical tube, add 2 ml of trypsin to the plate and incubate for 2 min. Use conditioned media to wash off cells and transfer to 50 ml tube. Centrifuge at 1000 rpm for 5 min.
4. Use 5 ml of media to wash poly-Lysine coated plates immediately before use.
5. Aspirate media and use 10 ml new RPMI media to re-suspend cell pellets and transfer to poly-Lysine coated plate.
6. For other cell lines, directly split them onto new plates.

B. Day 2

1. Collect conditioned media of 293sw cells into 50 ml conical tube inside hood. Centrifuge at 3000 rpm for 10 min.
2. Transfer conditioned media of BV2 cells to a fresh plate if necessary; aspirate all conditioned media from BV2 or U373 cells.
3. Transfer 8-10 ml of 293sw conditioned media to BV2 or U373 cells. Store 220 µl of conditioned media (duplicate wells and duplicate plates) for ELISA (on information sheet, ask 1:64 dilution of amyloid beta total, no dilution for amyloid beta 42).
4. Incubate for 24 hr, take 1.5 ml of conditioned media and spin at 14000 rpm for 5 min. Transfer 220 µl conditioned media (duplicate wells and duplicate plates) for ELISA (on information sheet, ask for 1:64 dilution of amyloid beta total, no dilution for amyloid beta 42).
5. Aspirate conditioned media and add 1 ml 20 mM EDTA in PBS. Collect cells and spin at 6000 rpm for 5 min. Store cell pellets at – 80°C.