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CALCIUM STAINING PROTOCOL

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ABSTRACT

Neurodegeneration due to neurotoxicity is one of the phenomena in temporal lobe epilepsy. Experimentally, hippocampal excitotoxicity process can occur due to kainic acid exposure, especially in the CA3 area. Neuronal death, astrocyte reactivity and increased calcium also occur in hippocampal excitotoxicity, but few studies have investigated immediate effect after kainic acid exposure. The organotypic hippocampal slice culture (OHSC) is a useful model for studying the neurodegeneration process, but there are still many protocol differences. In this study, minor modifications were made in the OHSC protocol.

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Calcium assay kit

1 We use Fluo-4 assay kit (calcium ab 228555) that provides a homogenous fluorescense-based assay for detecting the intracellular calcium mobilization

Material supplied in kit

2 Fluo-4 AM 1 vial
10x F127 Plus 1 bottle (10 mL)
HHBS (Hank's Buffer with 20 mM Hepes) 1 bottle (100 mL)

Reagent Preparation

3 Briefly centrifuge small vials at low speed prior to opening
Thaw all the kit components at room temperature before use
Fluo-4 AM stock solution : add 200 µL of DMSO into the vial of Fluo-4 AM and mix well
1x Assay buffer : make 1x assay buffer by adding 1 mL of 10x F127 plus into 9 mL of HHBS buffer and mix them well
Fluo-4 AM dye-loading solution : add 20 µL of Fluo-4 AM stock solution into 10 mL of 1x assay buffer and mix them well. This working solution is stable for at least 2 hours at room temperature

Assay procedure

4 Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate
Add 20 µL Fluo-4 AM dye-loading solution into the cell plate
Incubate the dye-loading plate in incubator with temperature 37^{o}C fo 1 h
Prepare the plate with HHBS
Run the calcium flux assay by monitoring the fluorescense intensity at Ex/Em = 490/525 nm with CLSM

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