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MAP-2 & GFAP 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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Sample Preparation

1 -Tissue slices were fixed using 4% PFA for 15 minutes
-Wash with PBS for 3 times (5 minutes each)
-Remove PBS and add 0,1% Triton X-100 for 30 minutes
-Wash with PBS for 3 times (5 minutes each)
-Add 10% BSA for 30 minutes at room temperature

Assay Procedure

2 -Incubate with primary antibody MAP-2 (1:500) and FDAP (1:500) overnight at temperature 4 ^{o}C.
-Wash with PBS for 3 times (5 minutes each)
-Incubate with secondary antibody for 1 h at room temperature
- Goat anti-Rabbit IgG-F (1:500) for MAP-2 (Santa Cruz Biotechnology sc-53805, Lot#K1109)
- Anti-mouse Rhodamine (1:500) for GFAP (Rockland, paint : 610-1002)
-Wash with PBS for 3 times (5 minutes each)
-Observe using CLSM with magnification of 100X and a wavelength of 488 nm (MAP-2) and 543 nm (GFAP)

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