

A SENSITIVE AND SELECTIVE ASSAY OF NEURONAL DEGENERATION IN CELL CULTURE

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Evaluation of neuronal death after toxin exposure in mixed neuron/glia cultures can be a challenging task. We have developed an assay to rapidly and selectively quantify neuronal death in mixed cultures based on the measurement of microtubule associated protein 2 (MAP2), a well characterized neuron-specific cytoskeletal protein. MAP2 immunoreactivity decreases as neurons degenerate, and we monitor this loss by an enzyme-linked fluorescence immunoassay. Briefly, cultured neurons are treated with toxin, fixed, and incubated with MAP2 antibody. The primary antibody is detected with a horseradish peroxidase (HRP)-conjugated secondary antibody in the presence of hydrogen peroxide and 10-acetyl-3-7-dihydroxyphenoxazine (ADHP). HRP catalyzes the conversion of the non-fluorescent ADHP to resorufin, which is highly fluorescent and detectable with a fluorescence platereader. We compared the sensitivity of our MAP2-based assay to the lactate dehydrogenase (LDH) efflux assay using glutamate (Glu) or kainate (KA) exposure to induce neuronal death. Increasing concentrations of either compound decreased MAP2 labeling and increased LDH release. However, the MAP2 method exhibits enhanced sensitivity over LDH by detecting neuronal death between a narrower range of neurotoxin. The MAP2 assay can detect difference in neuron death between 50 μ M and 100 μ M and 300 μ M Glu (5 min exposure). The magnitude of the signal change is also different. With the MAP2 assay, 300 μ M Glu induces a ~4X change in signal (to ~25% of untreated control levels), while the fold change for LDH efflux is ~1.6X of untreated control. This method can also detect neuroprotection in the presence of antioxidants after oxidant damage. The MAP2 assay can also detect neuron death in glial-enriched cultures after 100 μ M Glu (5 min exposure). This new method selectively detects neuronal death and survival by combining immunolabeling for a neuron-specific marker with the ease, sensitivity, and speed of an enzyme-linked fluorescence assay and will facilitate high-throughput screens of neurodegeneration and neuroprotective therapies.

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