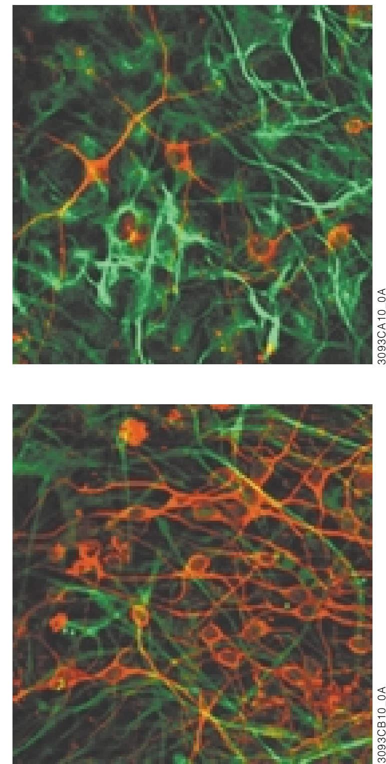
Specific Labeling of Neurons and Glia in Mixed Cerebrocortical Cultures

Thomas Gillessen, M.D., Franz Tokai and Kati Breiter
E-mail: Th.Gillessen@lrz.uni-muenchen.de
Institut fuer Pharmakologie und Toxikologie, Sanitaetsakademie der Bundeswehr, Germany

The specificity of staining neurons and glial cells was examined using Promega Anti-βIII Tubulin mAb (Cat.# G7121) directed against the neuronal β III-isoform of tubulin and Anti-GFAP pAb (Cat.# G5601) directed against glia-specific glial fibrillary acidic protein.

Introduction

Mixed cerebrocortical cultures taken from embryonic rats exhibit differentiated neurons and glia and are an important model system to study pharmacological and toxicological effects on cellular apoptosis. However, the use of a single immunocytochemical marker of apoptosis, such as terminal deoxynucleotidyl transferase-based dUTP nick-end labeling (TUNEL), without simultaneous specific staining of neurons or glia does not allow discrimination between neuronal and glial apoptosis. To specifically study apoptosis in neurons or glia in mixed primary cultures we looked for specific labeling of neurons and glia in cerebrocortical cultures. Using Promega's Anti-βIII Tubulin mAb (Cat.# G7121) directed against the neuronal βIII-isoform of tubulin and the Anti-GFAP pAb (Cat.# G5601) directed against glia-specific glial fibrillary acidic protein (GFAP), we examined the specificity of staining neurons and glial cells in these mixed cultures (Figure 1).



▲ **Figure 1.**
Detection of neuronal βIII-isoform of tubulin and GFAP in mixed cerebrocortical cultures. Panel A: Detection was performed using Anti-βIII Tubulin mAb (Cat.# G7121, *red*) followed by Anti-GFAP pAb (Cat.# G5601, *green*). Panel B: Detection was performed using Anti-GFAP pAb followed by Anti-βIII Tubulin mAb.

Cell Culture and Immunocytochemistry Pretreatment

Primary cerebrocortical cultures were obtained from E18 Sprague-Dawley rat embryos (Charles River, Sulzfeld, Germany). Cells were plated at a density of 1.8×10^5 cells/cm^2 onto nitric acid-washed and subsequently poly-D-lysine (100µg/ml) and laminine (1.1µg/ml) -coated glass coverslips. Cells were maintained at 37°C in 95% air/5% CO_2 in culture medium (solution of 79% DMEM, 10% fetal calf serum, 10% horse serum and 2mM L-glutamine). Half of the culture medium was replaced with fresh medium every 3–5 days in vitro (DIV). Glial cell proliferation was stopped by addition of 5µM cytosinarabinoside to the culture medium at DIV4. Experiments were performed after DIV11. For immunocytochemistry, cultures were fixed with 4% formalin at 37°C for 30 minutes and subsequently washed three times with PBS.

Labeling of Neuronal Tubulin Isoform and Glial Fibrillary Acidic Protein

Cells were preincubated with permeabilization buffer containing 0.15% Triton® X-100 in PBS and 10% horse serum for 1 hour at room temperature (RT). Following preincubation, cells were washed with PBS. Promega Anti-βIII Tubulin mAb was diluted in permeabilization buffer at a 1:200 dilution (from a 1mg/ml stock solution

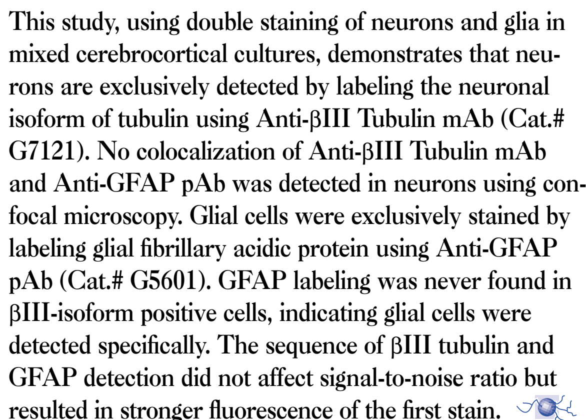
supplied in PBS). Primary antibody incubation was for 18 hours at RT. The antibody was detected using a Cy™3-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch) diluted in permeabilization buffer at a 1:250 dilution (from a 1.5mg/ml stock solution supplied in PBS).

Following labeling of the neuronal tubulin isoform, cells were preincubated with permeabilization buffer containing 0.15% Triton® X-100 in PBS and 10% goat serum for 1 hour at room temperature (RT). Anti-GFAP pAb was diluted in permeabilization buffer at a 1:100 dilution (from a 1mg/ml stock supplied in PBS). Incubation was for 18 hours at RT. Subsequently, the antibody was detected with a DTAF-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) diluted in permeabilization buffer at a 1:200 dilution (from a 2mg/ml stock solution). In some double-labeling experiments, GFAP labeling preceded tubulin labeling to study whether the signal-to-noise ratio of each detection is affected by the sequence.

Mounting with Antifade Reagents and Imaging

To prevent accelerated photobleaching from intense laser illumination, cell cultures were mounted using the ProLong Antifade Kit (Molecular Probes) and allowed to dry for 12 hours. Images were then taken with a Bio-Rad MRC 1024 laser-scanning confocal microscope with a 100mW argon ion laser (Bio-Rad) coupled to an Olympus BX-50WI microscope (Olympus). All experiments were performed using an Olympus 63x, 1.3 NA water immersion objective. Laser power was attenuated to 1–3% of maximal output power to prevent photobleaching.

Conclusions



This study, using double staining of neurons and glia in mixed cerebrocortical cultures, demonstrates that neurons are exclusively detected by labeling the neuronal isoform of tubulin using Anti-βIII Tubulin mAb (Cat.# G7121). No colocalization of Anti-βIII Tubulin mAb and Anti-GFAP pAb was detected in neurons using confocal microscopy. Glial cells were exclusively stained by labeling glial fibrillary acidic protein using Anti-GFAP pAb (Cat.# G5601). GFAP labeling was never found in βIII-isoform positive cells, indicating glial cells were detected specifically. The sequence of βIII tubulin and GFAP detection did not affect signal-to-noise ratio but resulted in stronger fluorescence of the first stain.

Acknowledgment

We thank Dr. Uwe Mohr, Promega GmbH, for his support.

Ordering Information

Product	Size	Cat.#
Anti-βIII Tubulin mAb	100µg	G7121
Anti-GFAP pAb	100µg	G5601