

## USING PROMEGA ANTIBODIES FOR NEUROSCIENCE RESEARCH

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We highlight a series of peer-reviewed articles in which Promega antibodies were used to follow molecular events in neural development or disease progression. The antibodies were used to study signaling cascades, cell-fate specification, and synaptic plasticity in a variety of model systems, ranging from primary cell cultures to nematodes to mammals.

### Introduction

Much of current neuroscience research seeks to understand the molecular events that underlie normal development or that lead to pathology in neurological diseases. Researchers must be able to follow specific molecules within cells and tissues and to detect modifications, such as protein phosphorylation of individual molecules, as they tease out the molecular pathways that govern cellular processes. The papers discussed here use Promega antibodies to study molecules involved in signaling cascades and to detect marker proteins specific to certain cell types.

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### Antibodies To Detect Phosphorylated Protein Kinases

Nervous system development requires both programmed genetic events and response to environmental cues. Cortical neurogenesis occurs during embryogenesis, but little is known about the signaling pathways that promote it. One study recently investigated the role of the MEK-C/EBP pathway in the transition of cortical progenitor cells to postmitotic neurons (1). The authors investigated levels of phosphorylated ERK (downstream substrate of MEK) in cortical progenitor cells isolated from mouse embryos at the beginning of neurogenesis. Western blot analysis indicated that ERK is phosphorylated in progenitor cells cultured in FGF2. When the cultured cells were infected with an adenovirus containing a dominant inhibitory form of MEK, Anti-ACTIVE® MAPK pAb (anti-phospho ERK, Cat.# V8031) was used in immunocytochemistry experiments to confirm that the construct did inhibit MEK activity. The authors investigated the effect of this inhibition on cell survival and differentiation and concluded activation of the MEK-C/EBP pathway fosters the neurogenic fate of cortical progenitors in this system.

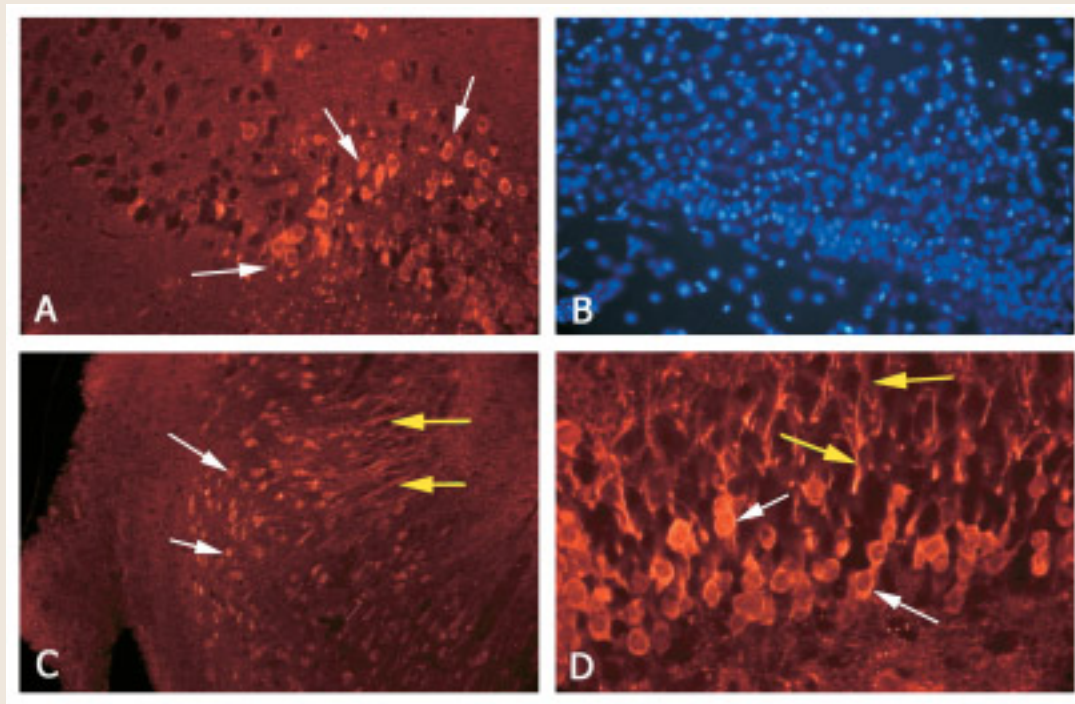
The development of the nervous system does not end after embryogenesis. The wiring of the visual cortex is shaped in great part by activity- or experience-dependent events. The protein kinase,  $\alpha$ CaM KII, plays a role in synaptic plasticity mechanisms. It initiates signaling toward the nucleus and dendrites of neurons, and this signaling is maintained by the autophosphorylation of the molecule. In the primary visual cortex, the transcription and translation of CaM KII is influenced by visual experience. In transgenic mice possessing a mutant form of  $\alpha$ CaM KII that cannot autophosphorylate, autophosphorylation of CaM KII is required for ocular dominance plasticity of the visual cortex (2). The authors used Anti-ACTIVE® CaM KII pAb (Cat.# V1111), to detect phosphorylated CaM KII by immunohistochemistry and by Western analysis in tissues isolated from these transgenic mice.

### Antibodies to Neurotrophic Factors and Receptors

Neurotrophic factors and their receptors are the molecules that sculpt the nervous system during development, direct its plasticity and remodeling, and determine the response of nervous tissue to injury. In response to injury of the central nervous system (CNS), the neurotrophin receptor (p75) is induced. In a recent article, Beattie *et al.* (3) report that p75 induction is required for death of oligodendrocytes in response to spinal cord injury and that proNGF is required for this p75 induction. The authors used Western analysis to monitor levels of pro and mature forms of NGF, BDNF, and NT-3 from the center of lesions after spinal cord injury. Promega Anti-Human NT-3 pAb (Cat.# G1651) and Anti-Human BDNF pAb (Cat.# G1641) were used to monitor the mature forms of these neurotrophic factors. No significant increases were detected for the amounts of pro or mature NT-3 or BDNF in response to spinal cord injury; however NGF levels did increase (both pro and mature). The proNGF molecule seems to be important in p75-activated apoptosis after spinal injury. Studies indicate that proNGF can induce p75-activated apoptosis among p75<sup>+/+</sup> oligodendrocytes in culture but not in p75<sup>-/-</sup> oligodendrocytes.

In a study of nervous system repair, researchers investigated the signaling pathway of myelin-associated glycoprotein (MAG; 4), which is one of several regeneration-inhibiting factors found in CNS myelin. These factors all show high affinity for the Nogo receptor (NgR), but NgR does not contain a cytoplasmic

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**Figure 1. Using Anti-ACTIVE® CaM KII to stain rat hippocampus.** All four images are taken from the same section. **Panel A.** Labeling in this region is specific for a subset of pyramidal neurons (arrows). **Panel B.** DAPI nuclear stain in the same field as A, further demonstrating the Anti-ACTIVE® CaM KII localization in a cellular subset. **Panel C.** Low-power image of a different area showing neuronal cell bodies (white arrows) and streaming dendrites (yellow arrows) labeled with Anti-ACTIVE® CaM KII. **Panel D.** High-power image of dentate granule cells showing intense labeling of neuronal cell bodies (white arrows) and dendrites (yellow arrows). Reprinted from Hoffman *et al.* (2001) *Cell Notes* 1, 4–7.

domain, and therefore presumably must work with a coreceptor. The Anti-Human p75 pAb (Cat.# G3231) was used in immunoprecipitation experiments that provided evidence to support the role of p75 as a coreceptor for NgR and to further establish the role of p75<sup>NTR</sup> in axon regeneration.

## β-Galactosidase Marker Antibody

Marker proteins are essential tools that enable scientists to follow proteins within cells and tissues. The *lac-z* gene is a standard marker in biology that can be used to tag cell-fate-specific proteins or to distinguish one population of cells from another based on their genetic content. In one recent study, the Anti-β-Galactosidase mAb (Cat.# Z3781) was used to detect a specific line of differentiated cells in the nematode, *Caenorhabditis elegans* (5). The authors were characterizing genes identified as suppressors of *C. elegans sel-12*, one of the nematode homologs of the human presenilin genes implicated in Alzheimer's disease. The precise roles of the presenilin genes in the cell are not completely understood, and this study sought to characterize genes that interact with the nematode presenilins. Mutations in *sel-12* presumably interfere with the LIN12/Notch signaling pathway that is critical for the correct specification of the pi cells, which form a membrane located between the uterus and the vulva in the

hermaphrodite nematode. The authors of the study identified pi cells based on the specific expression of a *lin-11::lac-Z* transgene, detected using the Anti-β-Galactosidase mAb.

Another study investigated the role of *Sonic Hedgehog* (*shh*) in organizing the developing mammalian neural tube. In this study, the authors used the Anti-β-Galactosidase mAb to distinguish modified embryonic stem cells from wildtype host cells in mouse chimeras (6). *Sonic Hedgehog*, one of three vertebrate genes related to the *Drosophila Hedgehog* gene, is involved in establishing the fates of neural progenitor cells along the dorsal-ventral and anterior-posterior axes of the developing neural tube. The authors investigated the role of *shh* in patterning in the neural tube by looking at the cell fates of *smoothened* (*Smo*) chimeras; *smoothened* is a gene required for *Hedgehog* signaling. Chimeric mice were created that were *smo*<sup>-/-</sup>, *smo*<sup>+/-</sup>, or *smo*<sup>+/+</sup> in a variety of genetic backgrounds. During the creation of the chimeras, β-galactosidase was used as a marker for genetically modified stem cells. ■

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## Citations

1. Menard, C. *et al.* (2002) An essential role for a MEK-C/EBP pathway during growth factor-regulated cortical neurogenesis. *Neuron* **36**, 597–610.
2. Taha, S. *et al.* (2002) Autophosphorylation of  $\alpha$ CaMKII is required for ocular dominance plasticity *Neuron* **36**, 483–91.
3. Beattie, M.S. *et al.* (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron* **36**, 375–86.
4. Wong, S.T. *et al.* (2002) A p75<sup>NTR</sup> and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nat. Neurosci.* **5**, 1302–7.
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6. Wijgerde, M. *et al.* (2002) A direct requirement for *Hedgehog* signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord. *Genes and Development* **16**, 2849–64.

## Ordering Information

Product	Size	Cat.#
Anti-Human NT-3 pAb	200 $\mu$ g	G1651
Anti-Human BDNF pAb	200 $\mu$ g	G1641
Anti-Human p75 pAb	200 $\mu$ g	G3231
Anti-ACTIVE <sup>®</sup> MAPK pAb, Rabbit (pTEpY)	40 $\mu$ l	V8031
Anti-ACTIVE <sup>®</sup> CaM KII pAb, Rabbit, (pT <sup>286</sup> )	40 $\mu$ l	V1111
Anti- $\beta$ -Galactosidase mAb*	100 $\mu$ g	Z3781
	2mg	Z3783

\*For Laboratory Use.

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## Technical Resources

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or the Antibody Assistant: [www.promega.com/techserv/tools/abasst](http://www.promega.com/techserv/tools/abasst)

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