Adenylate Cyclase III (ACIII) Chicken Polyclonal Antibody

CPCA-ACIII

Ordering Information
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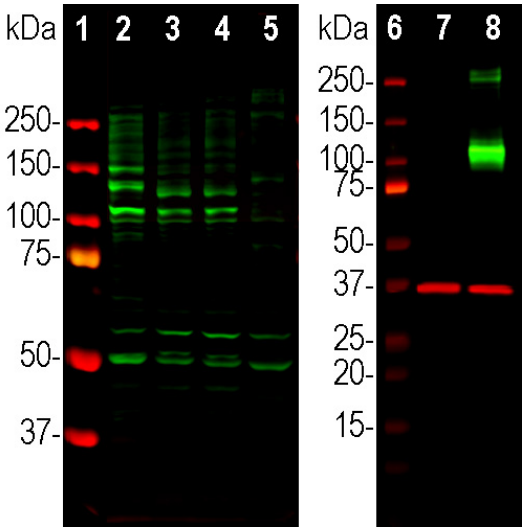
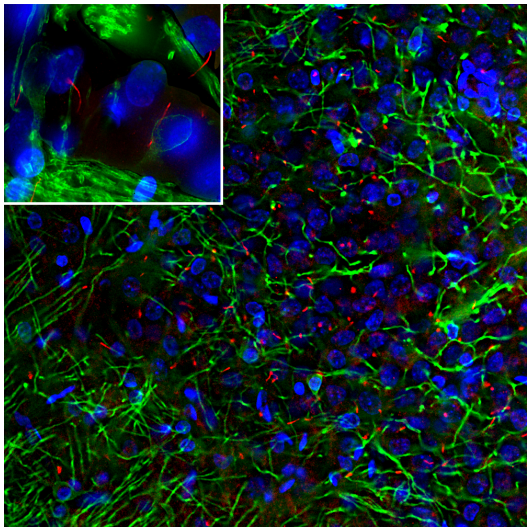
HGNC Name: ADCY3
UniProt: P21932
RRID: AB_2744500
Immunogen: C-terminal peptide of rat ACIII, PAAFPNGSSVTLPHQVVDNP with a Cys added to the N-terminus to allow coupling to KLH.
Format: Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3
Storage: Store at 4°C for short term, and at -20°C for longer term.
Recommended dilutions:
WB: 1:500-1:1,000, IF/ICC 1:5,000-1:10,000, IHC not recommended

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC	Chicken		~120kDa and above	Rt, Ms



Western blot analysis of different tissue lysates using chicken pAb to ACIII, CPCA-ACIII, dilution 1:1,000, in green. On the left blot: [1] protein standard, [2] rat hippocampus, [3] mouse hippocampus, [4] mouse frontal cortex, and [5] cow frontal cortex. CPCA-ACIII antibody detects variably glycosylated forms of ACIII protein with apparent molecular weights from ~120kDa and higher. On the right blot: [6] protein standard, [7] non-transfected HEK293 cells, and [8] HEK293 cells transfected with DNA expressing Myc-DDK tagged full length human adenylate cyclase III from the appropriate cDNA (ACIII). The strong band at about 130kDa demonstrates overexpression of the ACIII protein, and those over 250kDA double band presumably corresponds to heavily glycosolated or aggregated forms of ACIII. The same blot was simultaneously probed with mouse mAb to GAPDH, MCA-1D4, dilution 1:5,000, in red, which reveals the single GAPDH band at ~37kDa in both transfected and non-transfected cells.



Immunofluorescence analysis of rat cortex section stained with chicken pAb to adenylate cyclase III, CPCA-ACIII, dilution 1:10,000, in red and costained with mouse mAb to the myelin and oligodendrocyte marker CNP, MCA-1H10, dilution 1:1,000 in green. The blue is Hoechst staining of nuclear DNA. The ACIII antibody reveals neuronal cilia while the CNP antibody stains oligodendrocytes and the myelin sheath around axons.

Background: Trimeric G-proteins are a large and variable family of membrane receptors. On binding their specific ligand they activate specific members of the family of trimeric G-proteins which in turn activate other signalling enzymes. Adenylate cyclases are one of these downstream enzyme families which are activated by the GTP bound GαS subunits of trimeric G-proteins. Adenylate cyclases are responsible for the production of the important “second messenger” signaling molecule cyclic-AMP which in turn activates the cAMP dependent protein kinase. This kinase when activated phosphorylates numerous substrate molecules on serine or threonine residues and so alters their activity. There are several different adenylate cyclase genes and protein products with each have distinctly different distribution patterns in cells and tissues. The type III adenylate cyclase enzyme is specifically localized in the membranes surrounding neuronal cilia, and is activated by specific G-protein coupled receptors also located in cilia (1-5). Neuronal cilia express a variety of other receptors types and mediators of other signaling pathways and appear to function as a unique and complex neuronal sensory structure (1-5). For examples, the somatostatin 3 receptor, neuropeptide Y 2 receptor and melanin concentrating hormone receptor 1 are localized in neuronal cilia and the sonic hedgehog and Wnt signalling pathway act on neurons primarily through neuronal cilia (6). This antibody is an excellent marker of neuronal cilia in the brain and in cells in tissue culture and works in the same way as our rabbit polyclonal made against the same peptide (7). The CPCA-ACIII antibody was made against the extreme C-terminal peptide of rat ACIII, PAAFPNGSSVTLPHQVVDNP, amino acids 1125-1144 of the Genbank entry NP_570135.2. A cysteine residue was added to the N-terminus to allow coupling to MBS-activated keyhole limpet hemocyanin. The antibody works on mouse cells which express the same peptide and also on human cells, presumably because the corresponding peptide in the human AC3 sequence is the closely related peptide LATFPNGPSVTLPHQVVDNS. The antibody works well to identify neuronal cilia on human and rodent cells in IF and ICC, but is not recommended for IHC. We have also generated a mouse monoclonal and a rabbit polyclonal antibody to the same ACIII peptide, MCA-1A12 and RPCA-ACIII.

References:

1. Fuchs JL, Schwark HD. Neuronal primary cilia: a review. Cell Biol. Int. 28:111-8 (2004). 2. Louvi A and Grove EA. Cilia in the CNS: the quiet organelle claims center stage. Neuron 69:1046-60 (2011). 3. Singla V, Reiter JF. The primary cilium as the cell's antenna: signaling at a sensory organelle. Science 313:629-33 (2006). 4. Green JA, Mykytyn K. Neuronal Primary Cilia: An Underappreciated Signaling and Sensory Organelle in the Brain. Neuropsychopharm. 39:244–5 (2014). 5. May-Simera HL, Kelley MW. Cilia, Wnt signaling, and the cytoskeleton. Cilia 2;1:7 (2012). 6. Guemez-Gamboa A, et al. Primary cilia in the developing and mature brain. Neuron 82:511-21 (2014). 7. Guadiana SM, et al. Arborization of Dendrites by developing neocortical neurons is dependent on primary cilia and Type 3 adenylyl cyclase. J. Neurosci. 33:2626-38 (2013).

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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