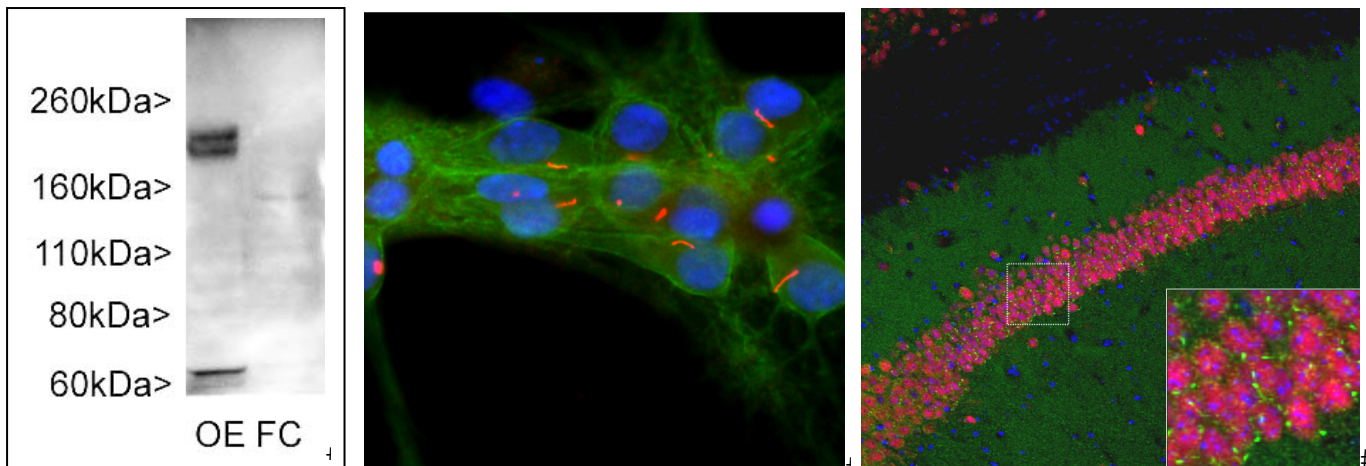


Catalogue RPCA-ACIII: Affinity Purified Polyclonal Antibody to Adenylate Cyclase III- Marker of Neuronal Cilia- ADCY3

The Immunogen: Adenylate cyclases are enzymes which interact with and are activated by the GTP bound, α subunits of trimeric G-proteins. Activated adenylate cyclases are responsible for the production of the important "second messenger" signaling molecule cyclic-AMP, which is generated from ATP. cAMP in turn activates cAMP-dependent protein kinase, which phosphorylates and alters the activity of other proteins. There are several different adenylate cyclase proteins with different distributions in cells and tissues. The type III adenylate cyclase enzyme is localized in the membranes surrounding the cilia in neurons, and as a result our antibody is an excellent marker of neuronal cilia in the brain and in cells in tissue culture. Neuronal cilia are interesting and complex structures which house important receptors mediating several kinds of signaling pathway (1, 2).

Adenylate cyclase type III is a large complex molecule of, in humans, 1145 amino acids with a deduced molecular weight of 129 kDa. The protein may be variably glycosylated, so that on SDS-PAGE and western blots it runs as a diffuse band of about 160 kDa in cortex and about 200kDa in olfactory epithelium (see below). The molecule has a complex structure, with 12 transmembrane domains and two cyclase domains. Each cyclase domain is immediately C-terminal to 6 transmembrane segments, but only the second, C-terminal cyclase domain is believed to be catalytically active.

Our antibody was raised against a 20 amino acid peptide identical to the C-terminus of rat ACIII, which is PAAFPNGSSVTLPHQVVDNP. The antibody works on human cells also, as the corresponding peptide in the human AC3 is the peptide LATFPNGPSVTLPHQVVDNS, which differs at only three amino acids. This antibody was generated in 2012, but already found utility as a definitive marker of neuronal cilia in a peer reviewed publication in the prestigious Journal of Neuroscience (3). In this paper the authors used our ACIII antibody to study the expression and localization of a variety of GFP, RFP and Cherry-tagged receptors which are targeted to cilia. The HGNC name for his protein is ADCY3.



Left: Western blots of rat olfactory epithelium (OE) and frontal cortex (FC). Our antibody labels bands at about 200 kDa in olfactory epithelium, a tissue which is rich in cilia. Fewer cilia are found in frontal cortex, and the protein is also less heavily glycosylated, so that a much less prominent band is seen at about 160 kDa. Data generated in the laboratory of Matt Sarkisian in the University of Florida. **Middle:** Confocal image of mixed rat neuron-glia cultures stained with our PRCA-ACIII (red) and our mouse monoclonal antibody to α II-spectrin

(**MCA-3D7**, green). Note the strong, clean and specific staining of neuronal cilia. Since α II-spectrin is specific for neurons in the CNS, the glial cells in this culture are not recognized by the spectrin antibody. The α II-spectrin antibody is also an excellent marker of neuronal plasma membranes. **Right:** Mouse brain sections (fixed by transcardial perfusion with 4% paraformaldehyde) stained with our RPCA-ACIII antibody (green) and our anti-Fox3/NeuN **MCA-1B7** antibody (red), showing specific labeling of cilia next to the pyramidal neurons in CA1 hippocampus region, but not in other part of the brain. Nuclei are labeled with Dapi (blue).

Antibody Characteristics: The antibody was raised against the peptide PAAFPNGSSVTLPHQVVDNP with a cysteine residue added to the N-terminus to allow coupling to Keyhole Limpet Hemocyanin. The peptide corresponds to the extreme C-terminus of the rat molecule. The antibody is provided in the form of affinity purified antibody at a concentration of 1 mg/mL with 50% glycerol/PBS. The antibody preparation contains 10 mM sodium azide preservative (Link to <http://www.encorbio.com/MSDS/azide.htm> for Material Safety Data Sheet).

Suggestions for Use: The antibody can be diluted to 1:500-1,000 for immunofluorescence staining and 1:5,000-10,000 for western blotting. On western blots look for a major bands at 160-200kDa, depending on the tissue.

Storage Instructions: Shipped on ice. Please store at 4°C for regular uses. For long term storage, please leave frozen at -20°C and avoid freeze/thaw cycles.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

Omim Link: <http://omim.org/entry/600291>.

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* 69:1046-1060 (2011).
3. Guadiana SM, Semple-Rowland S, Daroszewski D, Madorsky I, Breunig JJ, Mykytyn K, Sarkisian MR. Arborization of Dendrites by developing neocortical neurons is dependent on primary cilia and Type 3 adenylyl cyclase. *J Neuroscience* 33(6):2626-2638 (2013).

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