4949 SW 41st Blvd.
Suites 40 & 50
Gainesville, FL 32608
Tel: (352) 372 7022
Fax: (352) 372 7066
admin@encorbio.com

Catalogue# MCA-B630: Mouse Monoclonal Antibody to Rhodopsin: RHO

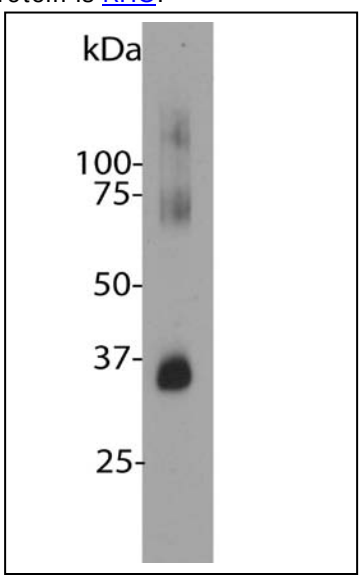
The Immunogen: Rhodopsin is the protein in the mammalian retina responsible for the light sensitivity of rod cells, which are responsible for vision in low light levels. Somewhat surprisingly, the rhodopsin protein turned out to be a typical member of the seven transmembrane G protein-coupled receptor (GPCR) superfamily. Whereas other GPCRs initiate signaling on binding a specific ligand, rhodopsin exists with a ligand already bound, specifically the vitamin A related substance retinal.

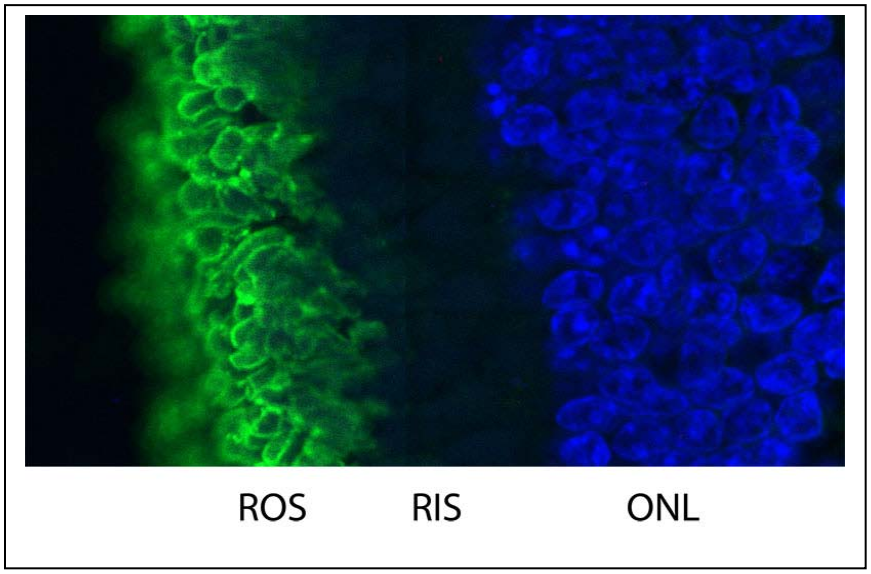
Retinal can exist in two isomeric forms, 11-cis and 11-trans retinal. In the dark, rhodopsin is associated with 11-cis retinal, but photons cause the 11-cis form to flip to the 11-trans form, and this causes an alteration in the structure of the rhodopsin making it catalytically active. Activated rhodopsin in turn activates the GTP binding protein G protein transducin by favoring the loss of GDP and the addition of GTP.

Transducin is a typical member of the family of heterotrimeric G proteins, and consists of an α and a βγ subunit. The α subunit is responsible for the GTP binding and the GTP bound form activates a phosphodiesterase (PDE) enzyme which hydrolyses cyclic GMP. This in turn increases the membrane potential of the rod cell and reduces the rate of synaptic signaling. So light stimulation actually results in a reduced rate of photoreceptor synaptic release. This information is transmitted through neurons of the retina to the visual centers of the brain (see review 1, 2).

Rhodopsin activity is shut off by phosphorylation under the influence of rhodopsin kinase, the activity of which results in binding of visual arrestin (a.k.a. arrestin-1 and S-antigen), which prevents rhodopsin from interacting with and activating more transducin molecules (3, 4). This basic signaling paradigm proved to be a prototype for understanding how other GPCRs function, as proteins similar to transducin, arrestin and rhodopsin kinase are found in these pathways.

MCA-B630 was raised against bovine outer segment discs and shows convincing staining for rhodopsin both on western blots and on sections of retina. The protein is concentrated in rod outer segments, and the MCA-B630 epitope has been mapped to the amino-terminus and is therefore on the exterior side of the membrane. The antibody has been used successfully in fluorescence activated cell sorting (FACS). The HGNC name for this protein is RHO.





Figures: Left: Blot of bovine retinal extracts probed with MCA-B630. The antibody stains a band corresponding to retinal rhodopsin at about 35 kDa. Bands about 70 kDa and 140 kDa are aggregated forms of rhodopsin. Note, due to the highly hydrophobic nature of rhodopsin, it important to avoid boiling samples containing this protein it in SDS-PAGE sample buffer, as this will result in even more extensive aggregation of the rhodopsin protein and appearance of more of this high molecular weight material. **Right:** High magnification confocal image of pig retinal section stained with MCA-B630 (Green). Rhodopsin is most abundant in the rod outer segments (ROS) of retina, clearly localized in rod membranes. The rod inner segments (RIS) and rod nuclei in the outer nuclear layer (ONL) are also seen in this image. Nuclear DNA was stained with DAPI (blue).

Antibody Characteristics: Antibody was raised in mouse against purified bovine rhodopsin. The MCA-B630 epitope is on the N-terminus and is so external to the cell. As a result the antibody can be used for fluorescence activated cell sorting. The antibody is purified and at a concentration of 1mg/mL. The preparation contains 10 mM sodium azide as a preservative. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: Try at dilutions of 1:1,000 for immunofluorescence. For Western blots try at 1:5,000. A suitable control tissue is rat, bovine or mouse retinal extract. The rhodopsin protein runs at about 35kDa on SDS-PAGE gels, and is a prominent component of retinal extracts. Due to the extreme hydrophobicity of the molecule, higher molecular weight bands of ~70 kDa and ~150 kDa are frequently seen on SDS-PAGE and western blots. This is less of a problem if samples are not boiled during sample preparation for SDS-PAGE..

References:

1. Molday RS. Photoreceptor membrane proteins, phototransduction, and retinal degenerative disease. The Frienwald lecture. Invest Ophthalmol Vis Sci. 39:2491-513 (1998).

2. Yau,KW . Phototransduction Mechanism in Retinal Rods and Cones. The Frienwald lecture. Invest Ophthalmol Vis Sci. 35:9-32 (1994).

3. Wilden U, Hall SW, Kühn H. Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. Proc Natl Acad Sci USA 83:1174-8 (1986).

4. Smith WC, Mc Dowell JH, Dugger DR, Miller R, Arendt A, Popp MP, Hargrave PA. Identification of regions of arrestin that bind to rhodopsin. Biochemistry Mar 38:2752-61 (1999).

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