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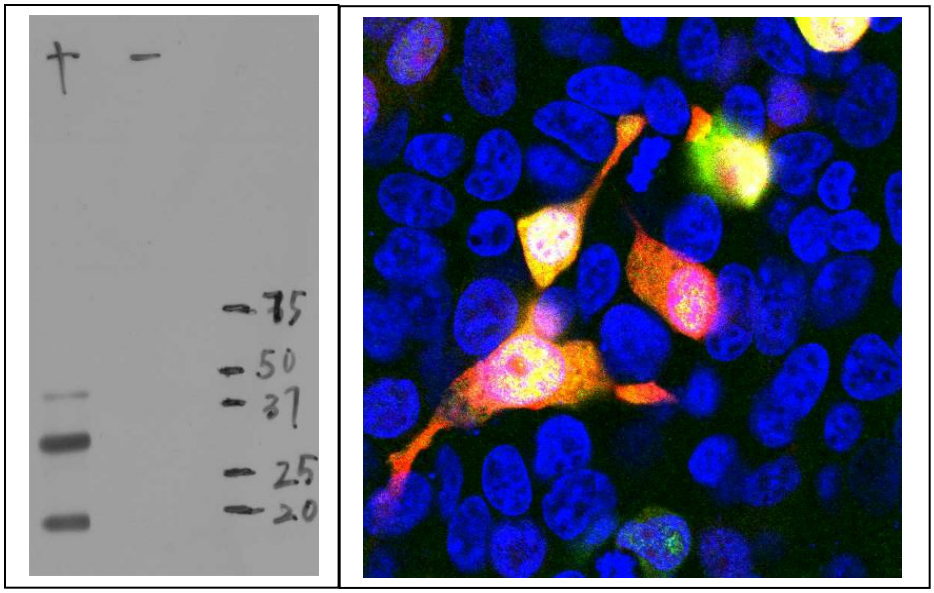
Catalogue# MCA-1C51: Monoclonal Antibody to mCherry

The Immunogen: mCherry is an engineered derivative of one of a family of proteins originally isolated from Cnidarians (jelly fish, sea anemones and corals). The prototype for these fluorescent proteins is Green Fluorescent Protein (GFP), which is a ~27 kDa protein isolated originally from the jellyfish *Aequoria victoria*. GFP was the basis of the 2008 Nobel Prize in Chemistry, awarded to Osamu Shimomura, Martin Chalfie and Roger Tsien, specifically "for the discovery and development of the green fluorescent protein, GFP". On expression from the GFP gene, GFP protein will fold correctly and fluoresce strongly, the development of fluorescence requires no cofactors except molecular oxygen. It can therefore be expressed in fluorescent form in essentially any prokaryotic or eukaryotic cell under aerobic conditions (1). As a result DNA encoding GFP can be fused to DNA encoding other proteins as a means to visualize the resulting fusion protein in live cells or animals. Engineered forms of GFP and relatives have been developed to monitor Calcium levels, protease activation and in a variety of other processes in real time. A whole range of GFP derivatives with different spectral properties have been developed, largely in the Tsien lab. The mCherry protein was derived from DsRed, a red fluorescent protein from so-called disc corals of the genus *Discosoma.* DsRed is a 223 amino acid ~28 kDa protein similar in size and properties to GFP, but, obviously, produces a red rather than a green fluorochrome. The original DsRed was engineered extensively in the Tsien lab to prevent it from forming tetramers and dimers and to modify and improve the spectral properties (2-4). The resulting monomeric protein is useful for applications such as Förster Resonance Energy Transfer (FRET, also known as Fluorescence Resonance Energy Transfer). Several further cycles of mutation, directed modification and evolutionary selection produced mCherry, which is monomeric and has an excitation maximum at 587 nm and emission maximum at 610 nm (5). We expressed the mCherry protein sequence shown in reference 5 in bacteria, purified out the mCherry and raised several mouse monoclonal antibodies. 1C51 was affinity purified and was found to stain a band of the expected size in HEK293 cells transfected with a vector designed to express mCherry which was developed in the Tsien lab and can obtained from Clontech.



Figures: Left: Blot of crude extract of HEK293 cells transfected with pFin-EF1-mCherry vector in lane labeled "+". The "-" lane is a blot of an equal amount of protein extract from untransfected HEK293 cells. MCA-1C51 binds a major band running at ~28 kDa corresponding to intact full-length mCherry. The two other bands are clearly processed forms of mCherry as they are not present in non-transfected HEK293 cells. **Right:** shows HEK293 cells transfected with mCherry and visualized in red. The cells were stained with MCA-1C51 in the green channel, and visualized using a confocal microscope. Transfected cells are yellow, showing overlap of the mCherry and the MCA-1C51 antibody. Untransfected HEK293 cells do not express Cherry and do not stain with the antibody, but their nuclei can be visualized using a DNA stain (blue). Blot and transfected cells courtesy of the Semple-Rowland lab at the University of Florida.

Antibody Characteristics: Antibody was raised against recombinant full length His tagged mCherry purified from *E. coli*. This antibody is IgG2a class and was affinity purified on Protein G and was diluted to a concentration of 1mg/ml. The preparation contains 10mM sodium azide as a preservative. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: Try at dilutions of 1:500 and higher for immunofluorescence. For western blots try at 1:2,000.

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

References:

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5. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nature Biotechnology 22:1567-1572 (2004).