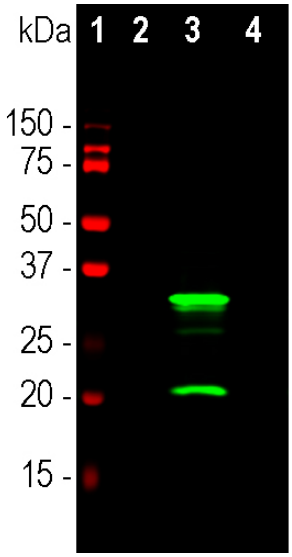
mCherry Mouse Monoclonal Antibody

MCA-5A6

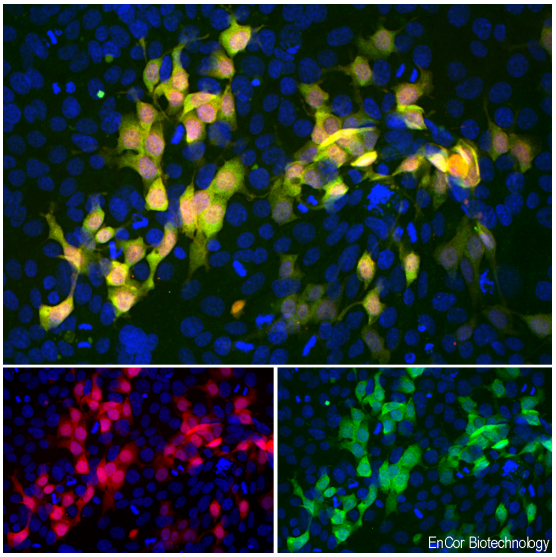
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *Not applicable*
UniProt: *D1MPT3*
RRID: *AB_2861221*
Immunogen: *Full length recombinant mCherry protein expressed in and purified from E. coli*
Format: *Purified at 1mg/mL in 50% PBS, 50% glycerol, 5mM NaN3*
Storage: *Store at 4°C short term, for longer term store at -20°C*
Recommended dilutions:
WB: 1:2,000 IF/ICC: 1:500. IHC: 1:2,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1 heavy, κ light	~28kDa	Not applicable



Western blot analysis of various HEK293 cell lysates using mouse mAb to mCherry, MCA-5A6, dilution 1:5,000, in green. [1] protein standard, [2] untransfected HEK293 lysate, [3] lysate of HEK293 cells transfected with mCherry-HA construct, and [4] lysate of HEK293 cells transfected with eGFP construct. As expected from our epitope mapping data, MCA-5A6 does not recognize eGFP. The major band at about 28kDa corresponds to the full length mCherry protein and the lower band at about 21kDa is an mCherry breakdown product.



Immunofluorescent analysis of HEK293 cells stably transduced with a lentiviral vector expressing mCherry-HA construct (red) and stained with mouse mAb to mCherry, MCA-5A6, dilution 1:500 in green. The blue is Hoechst staining of nuclear DNA. The MCA-5A6 antibody reveals the mCherry protein expressed only in transduced cells which appear golden in color. Untransduced cells expressing no mCherry are not recognized by the MCA-5A6 antibody and so only their nuclei are visible.

Background:

mCherry protein is derived from a natural product, DsRed, originally isolated as a red fluorescent protein from the coral of the genus *Discosoma* (1). As with other natural fluorescent proteins of Cnidarians (jelly fish, sea anemones and corals), the natural form of the protein forms stable tetramers in vivo. DsRed was engineered to improve its spectral properties and also prevent multimerization in the lab of Roger Tsien, where much work on fluorescent proteins was performed (2). Several further cycles of mutation, directed modification and evolutionary selection produced mCherry, which is monomeric and has an excitation maximum at 587nm and emission maximum at 610nm (3). The protein is widely used as a fluorescent tracer in transfection, transgenic, photobleaching and FRET type experiments. The prototype for these fluorescent proteins is Green Fluorescent Protein (GFP), which is a ~27kDa protein isolated originally from the jellyfish *Aequoria victoria* (4). The mCherry protein is similar in size and general structural properties to GFP (5,6), but, obviously, produces a red rather than a green fluorochrome.

The MCA-5A6 antibody was made against full length recombinant mCherry expressed in and purified from *E. coli*, EnCor product Prot-r-mCherry. MCA-5A6 antibody recognizes mCherry on western blots, in appropriate cells and sections, and does not react with GFP. The antibody has been epitope mapped to within the peptide IKQRLKLKDGGHYDAEVKTT, conserved in mApple and some other fluorescent proteins but not in GFP and others. We have tested this antibody on tdTomato, and found that it does not bind. However our alternate mouse monoclonal antibody to mCherry, MCA-1C51, binds equally well to mCherry and tdTomato. A sequence alignment of mCherry and GFP with epitope information is here. MCA-5A6 antibody can be used to verify the size of fusion constructs by western blotting, and to amplify the endogenous fluorescence of mCherry in transfected cells. We also supply rabbit, RPCA-mCherry, chicken, CPCA-mCherry, and goat polyclonal antibodies to this protein, GPCA-mCherry.

References:

1. Matz MV, et al. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat. Biotechnol. 17:969-73 (1999).
2. Baird GS, Zacharias DA, Tsien RY. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. PNAS 97:11984-9 (2000).
3. Chalfie M, et al. Green fluorescent protein as a marker for gene expression. Science 263:802-5 (1994).
4. Gross LA. et al. The structure of the chromophore within DsRed, a red fluorescent protein from coral. PNAS 97:11990-5 (2000).
5. Heikal AA. et al. Molecular spectroscopy and dynamics of intrinsically fluorescent proteins: coral red (dsRed) and yellow (Citrine). PNAS 97:11996-2001 (2000).
6. Shaner NC. et al. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat. Biotech. 22:1567-72 (2004).

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.

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.