EnCor Biotechnology Inc. Mouse Monoclonal Antibody

MCA-1C51

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

HGNC Name: Not applicable UniProt: DIMPT3 RRID: AB_2572309 Immunogen: Full length recombinant protein Format: Purified at 1mg/mL in 50% PBS, 50% glycerol, 5mM NaN3 Storage: Stable at 4°C for one year, for longer term store at -20°C Recommended dilutions: WB: 1:2,000 IF/IHC: 1:500.

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).

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).



Western blot analysis of HEK293 cell lysates, and recombinant protein solutions using mouse mAb to mCherry, MCA-1C51, dilution 1:1,000, in green [1] protein standard, [2] HEK293, [3] HEK293 cells transfected with mCherry-HA construct, [4] mCherry recombinant protein, [5] GFP recombinant protein, and [6] HEK293 transfected with GFP construct. Major band at about 30kDa corresponds to mCherry protein. MCA-1C51 antibody does not react with GFP protein. The same blot was simultaneously probed with chicken pAb to HSP60 CPCA-HSP60, dilution 1:5,000 in red which reveals band at 60kDa seen only in cell lysates.

isotype	Molecular Wt.
lgG2a heavy, κ light	~28kDa

Not applicable

Species Cross-Reactivity



Immunofluorescent analysis of HEK293 cells transfected with mCherry-HA, construct, in red, and stained with mouse mAb to mCherry, MCA-1C51, dilution 1:500, in green. The blue is Hoechst staining of nuclear DNA. MCA-1C51 antibody reveals mCherry protein expressed only in transfected cells which appear golden in color. Untransfected cells do not react with the antibody, as a result 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 Tsien lab, where much work on fluorescent proteins was performed (2). Roger Tsien, along with Martin Chalfie, and Osamyu Shinomura shared the 2008 Nobel prize in chemistry for the discovery and exploitation of Cnidarian fluorescent proteins. 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. As with GFP, mCherry becomes fluorescent due to intrinsic properties requiring only molecular oxygen and so can be readily expressed in a variety of systems.

The MCA-1C51 antibody was made against full length recombinant mCherry expressed in and purified from *E. coli*, EnCor product PROT-r-mCherry. The antibody recognizes mCherry strongly on western blots, in appropriate cells and sections and does not react with GFP. It does however react equally well with tdTomato, another derivative of DSRed, see data under the "additional info" tab. It can be used to verify the size of fusion constructs by western blotting and to amplify the endogenous fluorescence of mCherry in cells and tissues. We used the same recombinant immunogen to produce another mouse mAb, MCA-5A6, rabbit, RPCA-mCherry, chicken, CPCA-mCherry, and goat pAb to mCherry, GPCA-mCherry.

FOR RESEARCH USE ONLY. NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE.

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.

FOR RESEARCH USE ONLY. NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE.

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.