

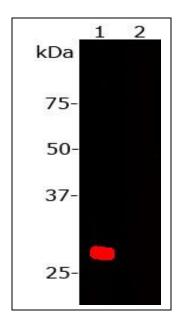
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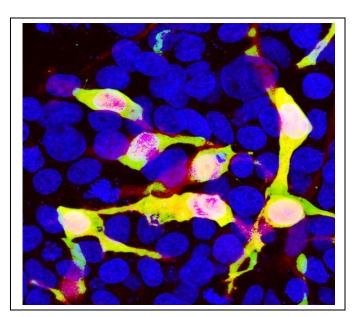
Catalogue# CPCA-mCherry: Chicken Polyclonal Antibody to mCherry

The Immunogen: mCherry is derived from proteins originally isolated from Cnidarians (jelly fish, sea anemones and corals), and is used as a fluorescent tracer in transfection and transgenic experiments. The prototype for these fluorescent proteins is <u>Green Fluorescent Protein</u> (GFP), which is a ~27 kDa protein isolated originally from the jellyfish *Aequoria victoria*. GFP was the basis of the <u>2008 Nobel Prize in Chemistry</u>, awarded to Osamu Shimomura, Martin Chalfie and Roger Tsien, specifically "for the discovery and development of the green fluorescent protein, GFP". GFP was shown to fluoresce on contact with molecular oxygen, requiring no other cofactors, and so can be expressed in fluorescent form in essentially any prokaryotic or eukaryotic cell.

The mCherry protein is derived from DsRed, a red fluorescent protein from so-called disc corals of the genus *Discosoma*. DsRed is 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 <u>Tsien lab</u> to prevent it from forming tetramers and dimers and to modify and improve the spectral properties (1-3). Several further cycles of mutation, directed modification and evolutionary selection produced mCherry, which has an excitation maximum at 587 nm and and emission maximum at 610 nm (4).

We expressed the mCherry protein sequence shown in reference 4 in bacteria, purified out the mCherry and raised a rabbit polyclonal antibody. This 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 obtained from Clontech. We also market a rabbit polyclonal antibody and a mouse monoclonal antibody to mCherry, specifically RPCA-mCherry and MCA-1C51.





Left: Western blot analysis of CPCA-mCherry. 1: HEK293 cells transfected with pFin-EF1-mCherry vector, courtesy of the Semple-Rowland lab at the University of Florida. There is a strong clean band at ~29 kDa. 2: HEK293 cells which were not transfected with this vector show no protein bands. CPCA-mCherry is at 1: 2,000 dilution. **Right:** HEK293 cells transfected in the same way and viewed in the confocal microscope. Most HEK293 cells are not transfected so only the nucleus of these cells can be visualized with a blue DNA stain. Cells which are transfected with Cherry are bright red. Staining with CPCA-Cherry is shown in Green. Green antibody staining is only seen cells which express Cherry, as expected, and the superimposition of the green and red results in an orange signal. Interestingly stronger Cherry staining is seen in the nucleus, possibly due to degradation of some Cherry molecules to release the low molecular weight Cherry fluorochrome.

Suggestions for use: Try at dilutions of 1:1,000 and higher for immunofluorescence. For western blots try at 1:2,000-5,000. The mCherry protein runs at \sim 29 kDa on SDS-PAGE gels.

Antibody Characteristics: Antibody was raised in chicken against recombinant full length His tagged mCherry purified from *E. coli*. This antibody is an IgY prep at a total protein concentration of 30 mg/mL. The preparation contains 0.02% sodium azide as a preservative. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

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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- 2. Gross LA, Baird GS, Hoffman RC, Baldridge KK, Tsien RY. The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc Natl Acad Sci U S A. 97:11990-5 (2000).
- 3. Heikal AA, Hess ST, Baird GS, Tsien RY, Webb WW. Molecular spectroscopy and dynamics of intrinsically fluorescent proteins: coral red (dsRed) and yellow (Citrine). Proc Natl Acad Sci U S A. 97:11996-2001 (2000).
- 4. 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).

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