

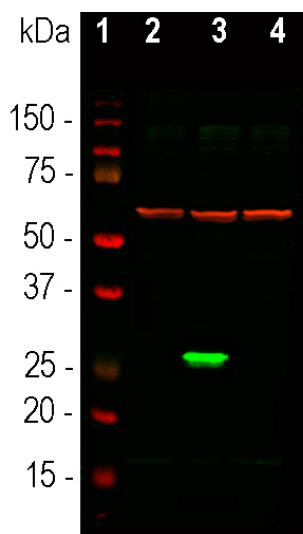
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HGNC Name: N.A.
UniProt: Q6YGZ0
RRID: AB_2572316
Immunogen: The prot-r-AcGFP recombinant protein purified from *E. coli*. The epitope is in the N-terminal 18 amino acids of the protein, the peptide MVSKGAELFTGIVPILIE, which is found in the Clontech and other GFP vectors
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN₃
Storage: Stable at 4°C for one year, for longer term store at -20°C
Recommended dilutions:
WB: 1:1,000-5,000 IF/IHC: 1:1,000-5,000

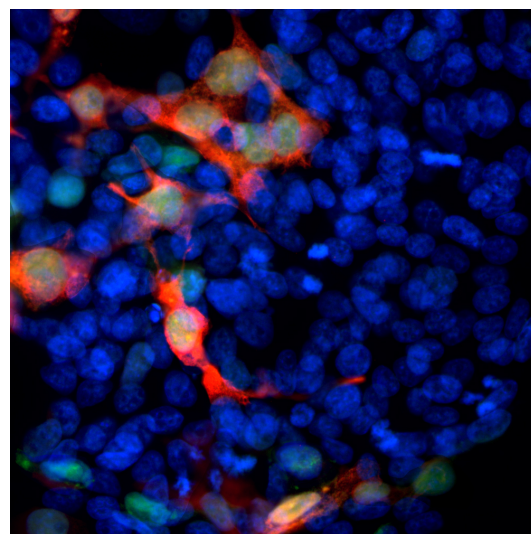
References:

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6. Heim R, Prasher DC, Tsien RY. Wavelength mutations and post-translational autooxidation of green fluorescent protein. *PNAS* 91:12501-04 (1994).
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Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgM	~27kDa	AcGFP, eGFP, not mCherry



Western blot analysis of HEK293 cell lysates using mouse mAb to GFP, MCA-3B11, in green, dilution 1:1,000: [1] protein standard, [2] non-transfected control cells, [3] cells transfected with a GFP construct, and [4] cells transfected with mCherry construct. The strong green band at ~27kDa corresponds to GFP protein detected only in cells transfected with GFP construct, the antibody does not recognize mCherry. The same blot was simultaneously probed with chicken pAb to HSP60, CPCA-HSP60, dilution 1:10,000, in red. The single band at 60kDa represents HSP60 protein expressed in all preparations.



Immunofluorescent analysis of transfected HEK293 cells with a GFP-construct in green stained with mouse mAb to GFP, MCA-3B11, dilution 1:1,000 in red. The blue is Hoechst staining of nuclear DNA. MCA-3B11 antibody reveals GFP protein expressed only in transfected cells, as a result cells are appeared in orange-golden color.

Background:

The **green fluorescent protein (GFP)** is a 27kDa protein isolated originally from the jellyfish *Aequorea victoria*. It has an endogenous fluorochrome activity with excitation maximum at 395nm and emission maximum at 509nm, which is similar to that of fluorescein (1,2). The GFP gene was sequenced and the origin of the fluorochrome by autocatalytic activity of certain amino acids was discovered (3,4). Much interest in GFP was generated when it was shown that fluorescence develops rapidly when the protein is expressed and requires only molecular oxygen and no other cofactors. As a result GFP can be expressed in fluorescent form in essentially any prokaryotic or eukaryotic cell (5). GFP has been engineered to produce a vast number of variously colored mutants including blue, cyan and yellow protein derivatives, BFP, CFP and YFP (6-9). GFP and other fluorescent proteins derived from other Cnidarians (jellyfish, coral and medusa) are widely used as tracers in transfection and transgenic experiments to monitor gene expression and protein localization *in vivo* and *in vitro*. The crystal structure of GFP was determined (7) which allowed amino acid modifications to improve spectral properties and prevent multimerization (8,9). The discovery GFP was the basis of the **2008 Nobel prize in chemistry**, specifically "for the discovery and development of the green fluorescent protein, GFP".

The MCA-3B11 antibody was made against a recombinant GFP construct originating from an *Aequorea* species which was engineered to improve spectral properties and prevent oligomerization (10). This form of GFP, referred to as AcGFP, is 94% identical to the eGFP developed by Tsien and coworkers and is the form of GFP inserted in the **Clontech/Takara pAcGFP and related expression vectors**. We epitope mapped this antibody to the N-terminal 18 amino acids of the AcGFP protein, the peptide MVSKGAELFTGIVPILIE, which is found in the Takara/Clontech and other GFP vectors and distinct from the sequence seen in other fluorescent proteins. The homologous region of eGFP is MVSKGEELFTGVVPILVE, and this antibody binds this peptide also. We also supply the immunogen, **PROT-AcGFP**. The antibody can be used to verify the expression, size and stability of both AcGFP and eGFP fusion proteins in western blotting experiments and to amplify GFP signals in tissues of transgenic animals. We also supply another mouse monoclonal antibody with a different isotype and rabbit, chicken, goat polyclonal antibodies to this protein, **MCA-1F1**, **RPCA-GFP**, **CPCA-GFP** and **GPCA-GFP**.

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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*.

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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.*