

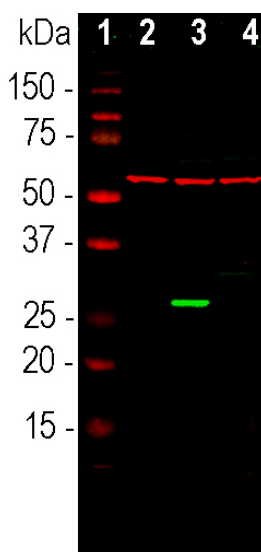
Ordering Information
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HGNC Name: N.A.
UniProt: Q6Y6Z0
RRID: AB_2572314
Immunogen: The prot-r-AcGFP recombinant protein purified from *E. coli*
Format: Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na₂S₂O₅
Storage: Store at 4°C for short term, for longer term at -20°C. Avoid freeze / thaw cycles.
Recommended dilutions:
WB: 1:1,000-5,000 IF/IHC: 1:1,000-5,000

References:

1. Shimomura O, Johnson FH, Saiga Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusa, *Aequorea*. *J. Cell. Comp. Physiol.* 3:223-39 (1962).
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3. Prasher DC, et al. Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* 111:229-33 (1992).
4. Cody CW, et al. Chemical structure of the hexapeptide chromophore of the *Aequorea* green-fluorescent protein. *Biochem.* 32:1212-8 (1993).
5. Chalfie M, et al. Green Fluorescent protein as a marker for gene expression. *Science* 263:802-5 (1994).
6. Heim R, Prasher DC, Tsien RY. Wavelength mutations and post-translational autooxidation of green fluorescent protein. *PNAS* 91:12501-04 (1994).
7. Ormo M, et al. Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* 273:1392-95 (1996).
8. Tsien RY. The green fluorescent protein. *Annu. Rev. Biochem.* 67:509-44 (1998).
9. Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. *Science* 296:913-6 (2002).
10. Gurskaya NG, et al. A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa *Aequorea coerulescens* and its fluorescent mutants. *Biochem. J.* 373:403-8 (2003).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Chicken		~27kDa	NA

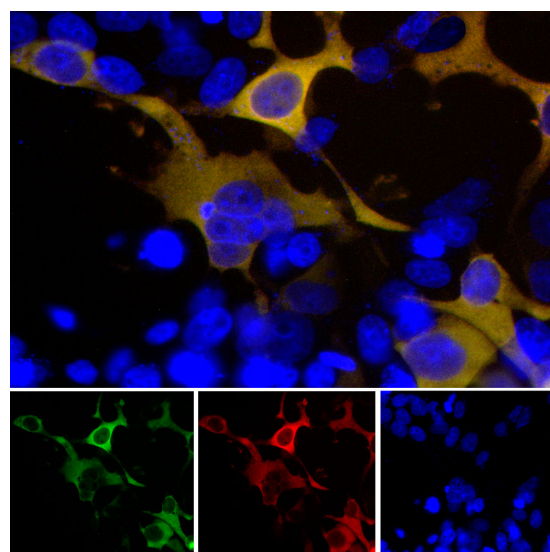


Western blot analysis of HEK293 cell lysates using goat pAb to GFP, GPCA-GFP, dilution 1:1,000, in green: [1] protein standard, [2] non-transfected control cells, [3] cells transfected with a GFP construct and [4] cells transfected with an mCherry construct. Strong band at ~27kDa corresponds to GFP protein detected only in cells transfected with GFP construct. This antibody does not recognize the mCherry protein. The 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.

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). GFP was the basis of the **2008 Nobel prize in chemistry**, specifically "for the discovery and development of the green fluorescent protein, GFP".

The CPCA-GFP 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 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 mouse monoclonal antibodies and rabbit and goat polyclonal antibodies to this protein, **MCA-3B11**, **MCA-1F1**, **RPCA-GFP** and **GPCA-GFP**.



Immunofluorescent analysis of transfected HEK293 cells with a GFP fusion protein construct, in green, and stained with chicken pAb to GFP, CPCA-GFP, dilution 1:1,000, in red. The blue is DAPI staining of nuclear DNA. The CPCA-GFP antibody reveals GFP protein expressed only in transfected cells, and as a result these cells appear golden yellow in color. Top, merged image, bottom individual channels.

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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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Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.*