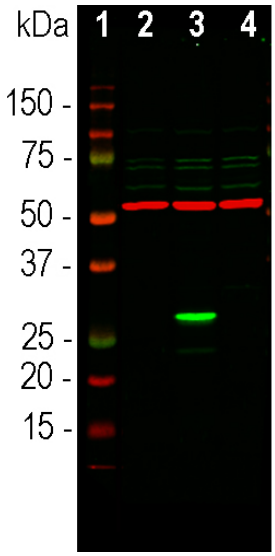
Green Fluorescent Protein Rabbit Polyclonal Antibody

RPCA-GFP

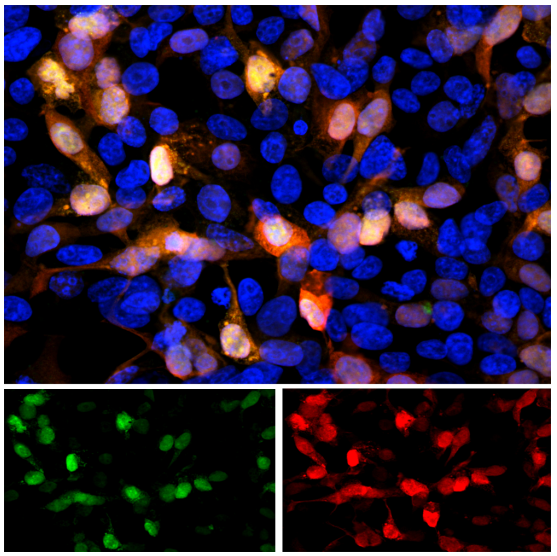
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *NA*
UniProt: *Q6YGZ0*
RRID: *AB_2572327*
Immunogen: *Recombinant AcGFP expressed in and purified from E. coli*
Format: *Affinity purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Stable at 4°C for one year, for longer term store at -20°C*
Recommended dilutions:
WB: 1:1,000-5,000. IF/ICC 1:2,000-5,000. IHC 1:2,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC	Rabbit		~27kDa	N.A.



Western blot analysis of lysates of transfected HEK293 using rabbit pAb to GFP, RPCA-GFP in green, dilution 1:2,000. [1] protein standard, [2] non-transfected control cells, [3] cells transfected with a GFP construct, and [4] cells transfected with mCherry construct. Strong 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 mouse mAb to β-tubulin, MCA-4E4, dilution 1:10,000, in red. The single band ~50kDa represents β-tubulin protein expressed in all preparations.



Immunofluorescent analysis of transfected HEK293 cells with a GFP construct, in green, and stained with rabbit pAb to GFP, RPCA-GFP, dilution 1:2,000 in red. The blue is Hoechst staining of nuclear DNA. The RPCA-GFP antibody reveals GFP protein expressed only in transfected cells, and as a result these cells appear orange-yellow in color.

Background:

The green fluorescent protein (GFP) is a 27kDa protein isolated originally from the jellyfish *Aequoria 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 *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 RPCA-GFP antibody was made against a recombinant GFP construct originating from an *Aequoria* 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 goat and chicken polyclonal antibodies to this protein, MCA-3B11, MCA-1F1, GPCA-GFP and CPCA-GFP.

References:

1. Shimomura O, Johnson FH, Saiga Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J. Cell. Comp. Physiol. 3:223–39 (1962).
2. Shimomura, O. Structure of the chromophore of Aequorea green fluorescent protein. FEBS Lett. 104:220–2 (1979).
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 autoxidation 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).

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.