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.

Immunofluorescence analysis of transfected HEK293 cells with a GFP-construct in green stained with goat pAb to GFP, GPCA-GFP, dilution 1:5,000. In red. The blue is Hoechst staining of nuclear DNA. The GPCA-GFP antibody reveals GFP protein expressed only in transfected cells, as a result transfected cells are appeared express both red and green signals and so appear an orange-golden color. Untransfected cells show neither signal.

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 cloned and 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 jellyfish, coral and other Cnidaria 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 (6) which allowed amino acid modifications to improve spectral properties and prevent multimerization (8,9). The 2008 Nobel prize in chemistry was awarded “for the discovery and development of the green fluorescent protein, GFP”.

The GPCA-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 pcGFP 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 chicken polyclonal antibodies to this protein, MCA-3B11, MCA-1F1, RPCA-GFP and CPA-GFP.