abcam

Product datasheet

Anti-GFP antibody [LGB-1] ab291

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Overview

Product name	Anti-GFP antibody [LGB-1]
Description	Mouse monoclonal [LGB-1] to GFP
Host species	Mouse
Specificity	This antibody recognizes all forms of GFP from Aquorea victoria (i.e. GFP, EGFP, YFP and CFP). See Abreview for CFP immunoprecipitation.
Tested applications	**Suitable for:** Flow Cyt, IP, ELISA, ICC/IF, WB
Species reactivity	**Reacts with:** Species independent
Immunogen	Recombinant full length protein corresponding to Escherichia coli GFP. Database link: P42212
Positive control	ICC/IF: GFP-transfected NIH3T3 cells

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	Preservative: None Constituents: 50% Glycerol, PBS, pH 7.2
Purity	Protein A purified
Clonality	Monoclonal
Clone number	LGB-1
Isotype	IgG1
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab291** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IP	★★★★☆	Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
ICC/IF	★★★★★	Use a concentration of 0.5 µg/ml.
WB	★★★★★	Use a concentration of 0.5 µg/ml. Detects a band of approximately 27 kDa (predicted molecular weight: 27 kDa).

Target

Relevance

Function: Energy-transfer acceptor. Its role is to transduce the blue chemiluminescence of the protein aequorin into green fluorescent light by energy transfer. Fluoresces in vivo upon receiving energy from the Ca^{2+} -activated photoprotein aequorin.

Subunit structure: Monomer.

Tissue specificity: Photocytes.

Post-translational modification: Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Ser-65 and Gly-67, and oxidation of Tyr-66 to didehydrotyrosine. Maturation of the chromophore requires nothing other than molecular oxygen.

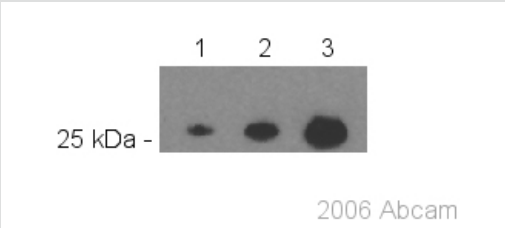
Biotechnological use: Green fluorescent protein has been engineered to produce a vast number of variously colored mutants, fusion proteins, and biosensors. Fluorescent proteins and its mutated allelic forms, blue, cyan and yellow have become a useful and ubiquitous tool for making chimeric proteins, where they function as a fluorescent protein tag. Typically they tolerate N- and C-terminal fusion to a broad variety of proteins. They have been expressed in most known cell types and are used as a noninvasive fluorescent marker in living cells and organisms. They enable a wide range of applications where they have functioned as a cell lineage tracer, reporter of gene expression, or as a measure of protein-protein interactions. Can also be used as a molecular thermometer, allowing accurate temperature measurements in fluids. The measurement process relies on the detection of the blinking of GFP using fluorescence correlation spectroscopy.

Sequence similarities: Belongs to the GFP family.

Biophysicochemical properties: Absorption: Abs(max)=395 nm
Exhibits a smaller absorbance peak at 470 nm. The fluorescence emission spectrum peaks at 509 nm with a shoulder at 540 nm.

Images

Images



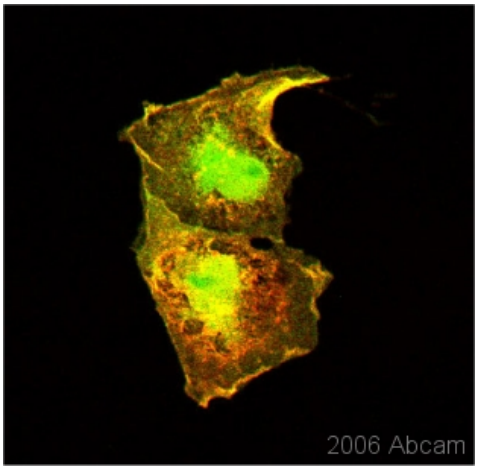
Western blot - Anti-GFP antibody [LGB-1] (ab291)

All lanes : Anti-GFP antibody [LGB-1] (ab291) at 0.5 µg/ml

Lane 1 : 5ng GFP
Lane 2 : 10ng GFP
Lane 3 : 25ng GFP

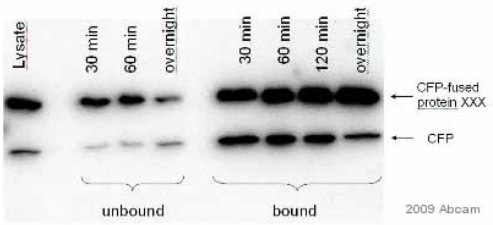
Secondary
All lanes : Sheep anti-mouse IgG HRP conjugate at 1/5000 dilution

Predicted band size: 27 kDa
Observed band size: 27 kDa



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [LGB-1] (ab291)

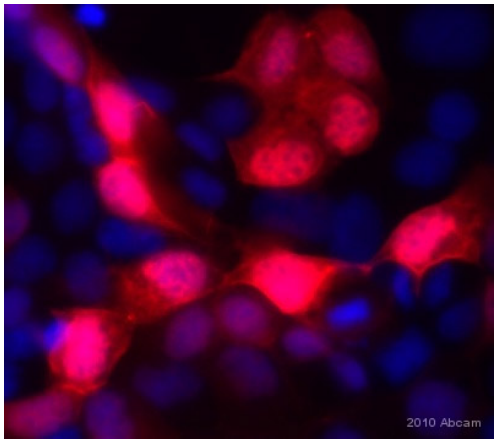
Paraformaldehyde fixed COS-7 cells expressing Myr-N15-PAK2-EGFP construct (Vilas et al.(2006) PNAS 103, 6542). Myr-N15-PAK2-EGFP fluorescence is shown in green. Indirect immunofluorescenct detection of N15-PAK-EGFP using ab291 monoclonal LGB-1 anti-GFP at 0.05 ug/ml with chicken anti-mouse secondary antibody conjugated to Alexa594 diluted 1/500 is shown in red. Myr-N15-PAK2-EGFP is localized to membrane ruffles and perinuclear vesicular structures (likely Golgi,TGN or late endosomes).



Immunoprecipitation - Anti-GFP antibody [LGB-1] (ab291)

This image was kindly supplied by Dr Lindsay Tulloch by Abreview

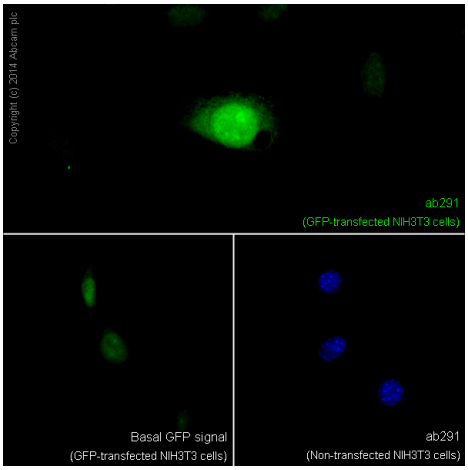
ab291 at 6.7µg/mg lysate.
HEK293 Cell lysate at 300µg.
Transfected with CFP-fused protein XXX in pECFP vector.
Immunoprecipitation step using Protein G.



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [LGB-1] (ab291)

This image is courtesy of an Abreview submitted by Vladimir Milenkovic

ab291 staining GFP in Dog MDCKII cells transfected with GFP by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% TX100 and blocked with 5% serum for 20 minutes. Samples were incubated with primary antibody (1/250 PBS + 0.1% TX100 + 1% goat serum) for 16 hours at 4°C. An Alexa Fluor®546-conjugated Goat anti-mouse IgG polyclonal (1/500) was used as the secondary antibody. DAPI was used to stain nuclei. ab291 was used to assess electoporation efficiency of double transfected MDCKII cells.



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [LGB-1] (ab291)

ab291 staining GFP in GFP-transfected NIH3T3 cells. The cells were fixed with 4% formaldehyde (10min) and then blocked in 1% BSA / 0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab291 at 1/200 dilution overnight at +4°C followed by incubation with ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488), for 1 hour, at 1µg/ml.
Under identical experimental conditions, when compared to the basal level of GFP expression in transfected NIH3T3 cells, the cells upon which ab291 was applied gave a stronger signal in the 488 channel, indicating that ab291 is binding to GFP and therefore eliciting signal amplification.
ab291 was also applied to non-GFP-transfected NIH3T3 cells, which produced no positive staining, indicating specificity for GFP. Nuclear DNA was labelled with 1.43µM DAPI (blue).

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