abcam

Product datasheet

Anti-GFP antibody ab6673

16 Abreviews 212 References 10 Images

Overview

Product name	Anti-GFP antibody
Description	Goat polyclonal to GFP
Host species	Goat
Specificity	Anti-GFP assayed by ELISA for direct binding of antigen recognizes wild type, recombinant and enhanced forms of GFP.
Tested applications	**Suitable for:** WB, IP, ELISA, ICC/IF, IHC-P, IHC-FrFl, IHC-Fr
Species reactivity	**Reacts with:** Species independent
Immunogen	Fusion protein corresponding to *Aequorea victoria* GFP aa 1-246. Database link: P42212
Positive control	IHC: E5.5 Hex-GFP transgenic mouse embryo. WB: HeLa cells. Green Fluorescent protein.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.42% Potassium phosphate, 0.87% Sodium chloride
Purity	Affinity purified
Purification notes	This product was prepared from monospecific antiserum by immunoaffinity chromatography using Green Fluorescent Protein (*Aequorea victoria*) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab6673** 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
WB	★★★★★	1/400 - 1/2000. (for immunoprecipitated GFP, see Abreview).
IP		Use at an assay dependent concentration.
ELISA		1/40000. This antibody can be used to detect GFP by ELISA (sandwich or capture) for the direct binding of antigen and recognizes wild type, recombinant and enhanced forms of GFP.
ICC/IF		1/500.
IHC-P	★★★★☆	1/200 - 1/1000.
IHC-FrFl		Use at an assay dependent concentration.
IHC-Fr	★★★★☆	1/200 - 1/1000.

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.

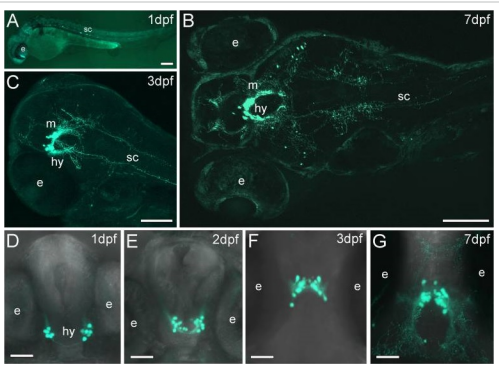
Biotechnological use: Green fluorescent protein has been engineered to produce a vast number of variously colored mutants, fusion proteins, and biosensors. Fluorescent proteins and its mutated allelic forms, blue, cyan and yellow have become a useful and ubiquitous tool for making chimeric proteins, 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

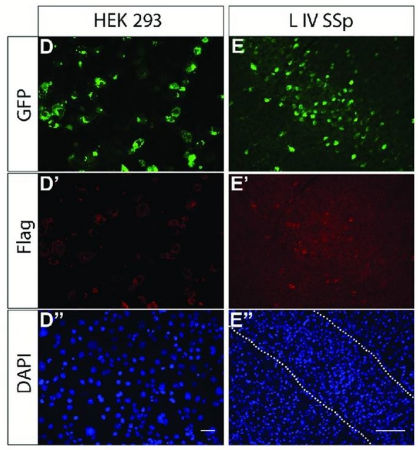


Immunohistochemistry - Free Floating - Anti-GFP antibody (ab6673)

Suarez-Bregua et al PLoS One. 2017 Oct 17;12(10):e0186444. doi: 10.1371/journal.pone.0186444. eCollection 2017. Fig 1.

Pth4:eGFP transgenic zebrafish embryos at 1 and 2 dpf were fixed with 4% PFA and washed in PBST. They were then washed in PBDT (1% BSA, 1% DMSO, 0.1% Triton X-100 in PBS, pH 7.4), blocked in 10% normal goat serum/PBDT, and incubated overnight at 4°C with primary antibodies to HuC/D (1/100) and GFP (1/400, Abcam ab6673). Further PBST washes and blocking were followed by secondary antibodies overnight at 4°C. Hoechst 34580 was added to stain nuclei (1/2500). After further PBDT and PBS washes, embryos were mounted for confocal imaging.

Abbreviation: e, eye; hy, hypothalamus; m, midbrain; sc, spinal cord. Scale bars: 100 μm (A-C) 50 μm (D-G).



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody (ab6673)

Borkowska et al PLoS One. 2016 May 31;11(5):e0156082. doi: 10.1371/journal.pone.0156082. eCollection 2016. Fig 5.

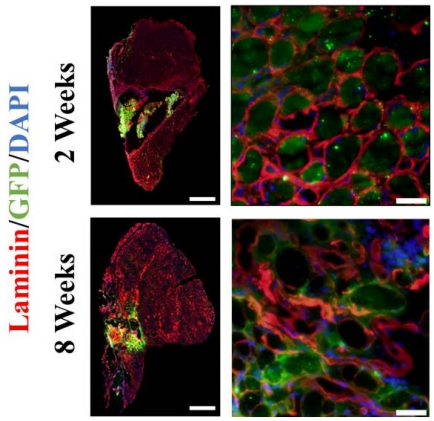
***In utero* electroporation of Disc1 and Disc1-100P constructs into wild-type neocortex and analysis at P21.**

(Panels D-E") Expression of the constructs was assessed.

(Panels D-D") 2 days after transfection *in vitro.*

(Panels E-E") at P21 *in vivo.*

Immunochemistry for FLAG and GFP showed that constructs encoding either WT Disc1, the Disc1-100P variant, or GFP alone, expressed these protein species in transfected HEK-293 cells *in vitro* (Fig 5D–5D") and in P21 postmitotic cortical neurons *in vivo* (Fig 5E–5E")



Immunohistochemistry (Frozen sections) - Anti-GFP antibody (ab6673)

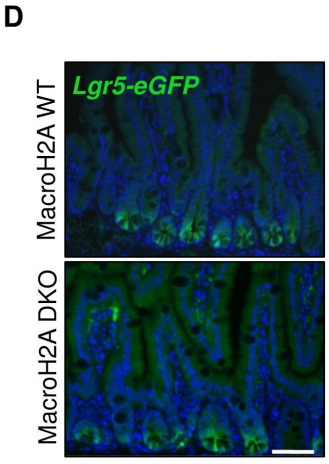
Goldman et al PLoS One. 2018 Jan 12;13(1):e0191245. doi: 10.1371/journal.pone.0191245. eCollection 2018. Fig 5.

Immunofluorescence for assessment of GFP$^+$ myofibers in rat tissue.

VML affected muscle from the 50% MG + HA+LMN group were probed for the presence of GFP. GFP+ fibers were detected in a qualitatively similar magnitude at both 2 and 8 weeks post-injury indicating viable engraftment of donor derived muscle progenitor cells. Scale bars are 1mm for whole mount images, 50 µm for regions of interest.

A portion of the TA muscle from the defect region was embedded in a talcum-based gel, frozen in 2-methylbutane, and supercooled in liquid nitrogen. Cryosections (8 µm) were prepared and stained using standard protocols for hematoxylin & eosin.

ab6673 used at a 1/100 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab6673)

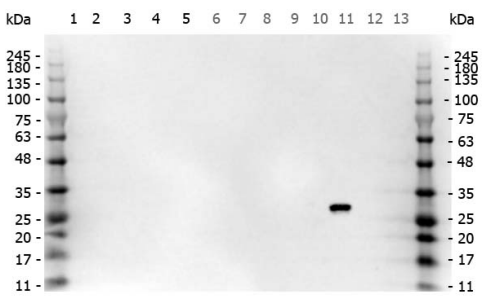
Cedeno et al PLoS One. 2017 Sep 21;12(9):e0185196. doi: 10.1371/journal.pone.0185196. eCollection 2017. Fig 3.

Mouse small intestines were washed with DPBS and fixed overnight at 4°C in Zinc formalin. Following sectioning and tissue deparaffanization, antigen retrieval was performed with 10mM Tris base (pH 9.0) buffer using a pressure cooker.

For immunohistochemistry, sections were quenched of endogenous peroxidases by 3% H_2O_2, and sequentially blocked with Avidin D, biotin, and protein blocking reagents. Primary antibody incubation was conducted at 4°C overnight. Secondary biotinylated antibody was added at a dilution of 1/200, and incubated 2 hours at room temperature. Finally, sections were stained according to the ABC peroxidase protocol and counterstained with hematoxylin.

ab6673 used at a 1/200 dilution.

Panel D: Representative anti-eGFP immunofluorescence of macroH2A WT and DKO jejunum counterstained with DAPI (blue).



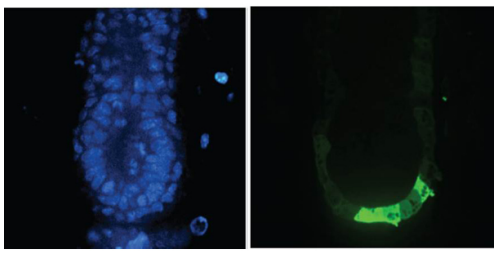
Western blot - Anti-GFP antibody (ab6673)

All lanes : Anti-GFP antibody (ab6673) at 1 µg/ml (o/n at 4degC)

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) lysate at 10 µg
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) lysate at 10 µg
Lane 3 : CHO/K1 lysate at 10 µg
Lane 4 : MDA-MB-231 (Human breast adenocarcinoma cell line) lysate at 10 µg
Lane 5 : A431 (Human epidermoid carcinoma cell line) lysate at 10 µg
Lane 6 : Jurkat (Human T cell leukemia cell line from peripheral blood) lysate at 10 µg
Lane 7 : NIH/3T3 (Mouse embryo fibroblast cell line) lysate
Lane 8 : E-coli HCP control, 50 ng
Lane 9 : FLAG Positive control lysate at 10 µg
Lane 10 : Red fluorescent protein, 50 ng
Lane 11 : Green fluorescent protein, 50 ng
Lane 12 : Glutathinoe-S-Transferase protein, 50 ng
Lane 13 : Maltose Binding protein, 50 ng

Secondary
All lanes : Peroxidase goat secondary antibody, 60 min at RT at 1/30000 dilution

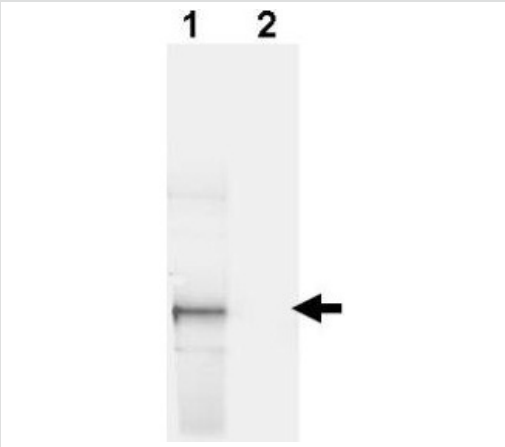
Blocking Buffer: 1% Casein-TTBS for 30 min at RT.



Immunohistochemistry - Anti-GFP antibody (ab6673)

E5.5 Hex-GFP transgenic mouse embryo stained for GFP using ab6673 at 1/500 dilution. Secondary antibody is a fluorochrome conjugated anti-goat IgG secondary antibody at 1/10,000 for 45 min at RT.

Staining: GFP as green fluorescent signal with DAPI blue counterstain.



Western blot - Anti-GFP antibody (ab6673)

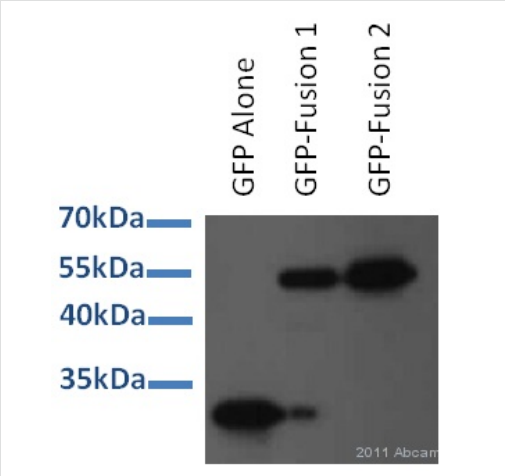
All lanes : Anti-GFP antibody (ab6673) at 1 µg/ml

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cells
Lane 2 : Mock transfected HeLa cell lysate

Lysates/proteins at 35 µg per lane.

Secondary
All lanes : IRDye® 800 conjugated Donkey-a-Goat IgG [H&L] at 1/2500 dilution

Additional bands at: 33 kDa. We are unsure as to the identity of these extra bands.



Western blot - Anti-GFP antibody (ab6673)
This image is courtesy of an anonymous abreview.

All lanes : Anti-GFP antibody (ab6673) at 1/1000 dilution

Lane 1 : MRC5VA lung fibroblast whole cell lysate overexpressing EGFP alone
Lanes 2-3 : MRC5VA lung fibroblast whole cell lysate overexpressing an EGFP fusion protein

Lysates/proteins at 15 µg per lane.

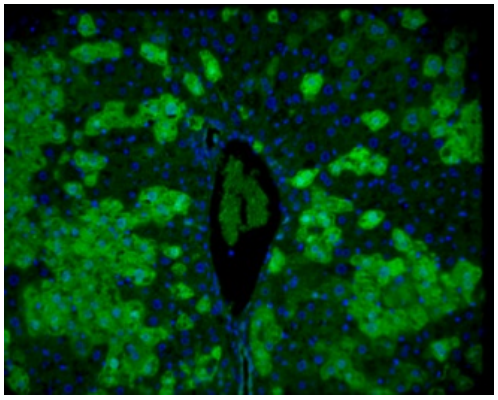
Secondary
All lanes : HRP-conjugated anti-goat polyclonal at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 27,55 kDa
why is the actual band size different from the predicted?

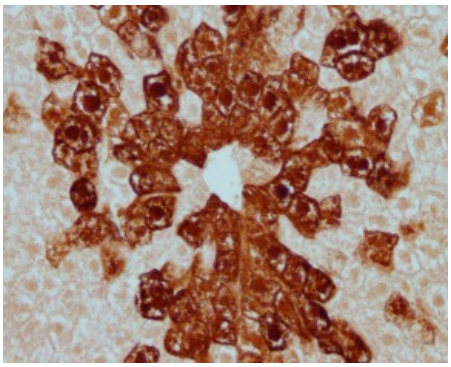
Exposure time: 5 seconds



Immunofluorescence of TGN mouse liver labeling GFP on hepatocytes with ab6673.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab6673)

This image is courtesy of Bart Rountree



Immunohistochemistry of GFP transgenic mouse liver labeling GFP with ab6673.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab6673)

This image is courtesy of Jeff Klein

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