abcam

Product datasheet

Anti-GFP antibody ab290

★★★★★ 178 Abreviews 3088 References 11 Images

Overview

Product name	Anti-GFP antibody
Description	Rabbit polyclonal to GFP
Host species	Rabbit
Specificity	Anti-GFP antibody (ab290) is a highly versatile antibody that gives a stronger signal than other anti-GFP antibodies available. On Western blot the antibody detects the GFP fraction from cell extracts expressing recombinant GFP fusion proteins and has also been shown to be useful on mouse sections fixed with formalin. In Immunocytochemistry, the antibody gives a very good signal on recombinant YES-GFP chimeras expressed in COS cells (McCabe et al. 1999 and figure below). It is routinely used in Immunoprecipitation (IP) and IP-Western protocols and has been used successfully in HRP Immunohistochemistry at 1:200 on whole-mount mouse embryos. **GFP antibody is reactive against all variants of *Aequorea victoria* GFP such as S65T-GFP, RS-GFP, YFP, CFP, RFP and EGFP.**
Tested applications	**Suitable for:** ELISA, IHC-Fr, ICC, IHC-P, IP, WB, IHC-FoFr, IHC-FrFl, Electron Microscopy
Species reactivity	**Reacts with:** Species independent
Immunogen	Recombinant full length protein corresponding to GFP. Green fluorescent protein (GFP) from Aequorea victoria. Database link: **P42212**
Positive control	The Recombinant A. victoria GFP protein (**ab84191**), any other purified recombinant GFP, any cell line confirmed to overexpress GFP. ICC: NIH3T3, U2OS and glandular stomach cells. IHC: Mouse brain and dog heart tissue. WB: Sample: COS7 and LNCaP whole cell lysate - transfected with GFP-Eml4.
General notes	The total IgG concentration has been determined to be 5 mg/mL. The specific IgG concentration is unknown. This product should be kept refrigerated at all times whilst in short term storage. Using sterilised equipment will reduce the risk of bacterial contamination. Anti-GFP antibody (**ab6556**) is the purified version of this antibody (see Related Products). The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: 1.25% Sodium chloride
Purity	Whole antiserum
Purification notes	This antibody is provided as whole antiserum. It is not possible to determine the exact antibody concentration, since whole serum contains many other host serum proteins besides the antibody of interest.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab290 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
ELISA		Use at an assay dependent concentration.
IHC-Fr	★★★★★ **(8)**	Use at an assay dependent concentration. Reported to work at dilutions up to 1/3000. Use secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab15077**).
ICC	★★★★★ **(2)**	1/200 - 1/1000. We recommend **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081)** secondary antibody.
IHC-P	★★★★★ **(24)**	1/500 - 1/1000. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.
IP	★★★★★ **(24)**	Use at an assay dependent concentration. Use at 1µl per 10cm tissue culture dish (use 10µl protein A agarose CL4B to precipitate the immune complex).
WB	★★★★★ **(69)**	1/1000 - 1/2500.

Application	Abreviews	Notes
IHC-FoFr	★★★★★ (5)	1/200 - 1/500.
IHC-FrFl	★★★★★ (2)	Use at an assay dependent concentration.
Electron Microscopy		1/1000 - 1/4000.

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.

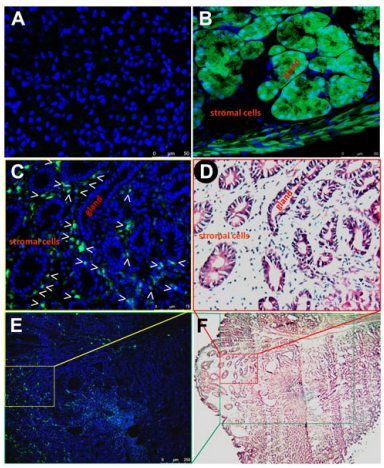
Post-translational modification: Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Ser-65 and Gly-67, and oxidation of Tyr-66 to didehydrotyrosine. Maturation of the chromophore requires nothing other than molecular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

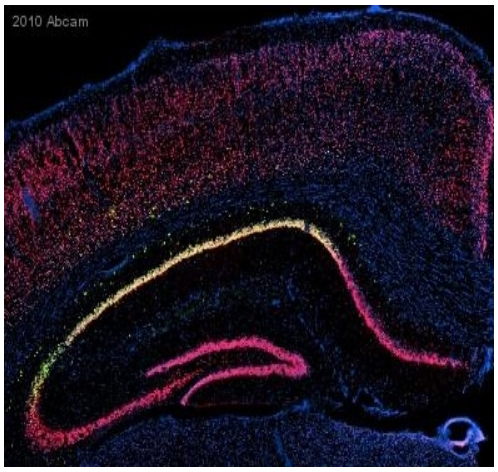
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab290)

Bone marrow-derived infiltrating cells in the stromal tissue of gastric intraepithelial tumor traced by GFP direct fluorescence.

(**A**) Normal tissues of the glandular stomach of a regular GFP(−) control mouse. (**B**) Normal tissues of the glandular stomach of a GFP(+) transgenic control mouse; (**C, E, D, F**) An induced gastric intraepithelial neoplasia (GIN) in a bone marrow transplanted mouse. GFP(+) BMDCs tracked with direct fluorescence localized in the GIN stromal tissue are shown in **C** and **E**. The same GIN lesion slide stained by H&E after the fluorescence observation are shown in **D** and **F**. DAPI (**A–C** and **E**) and hematoxylin (**D** and **F**) are used to visualize nuclei, respectively. Locations of the images **C** and **D** in the images **E** and **F**, and the image **E** in the image **F** are marked in the corresponding color. The gastric glands and stromal cells are also labeled.

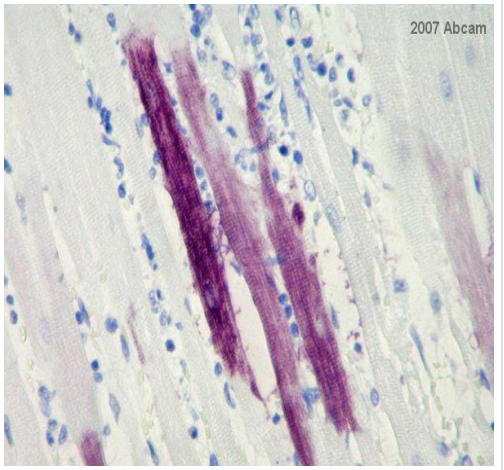


Immunohistochemistry - Free Floating - Anti-GFP antibody (ab290)

This image is courtesy of an Abreview submitted by Judith Kranz

Immunohistochemistry (Free Floating) analysis of mouse brain tissue sections labelling GFP with ab290. Tissue was fixed with 4% PFA, frozen 30 µm sections were blocked for 1 hour at room temperature with 10% normal goat serum + donkey anti-mouse IgG Fab fragments (0.1 mg/ml). Sections were incubated with the primary antibody at a dilution of 1/1000 in TBS + 0.25% Triton-X for 16 hours at 4°C. A Cy2®-conjugated donkey anti-rabbit IgG (H+L) at a dilution of 1/200 was used as the secondary antibody.

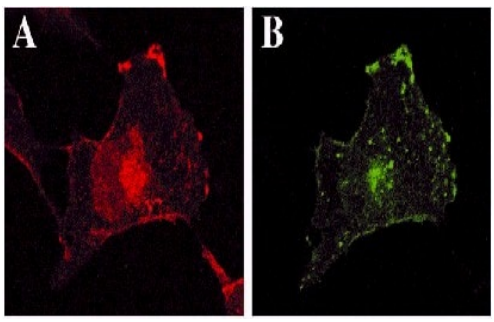
Image shows anti-NeuN (red), DAPI (blue), and anti-GFP staining of GFP-cre (green, yellow with NeuN colocalization).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab290)

This image is courtesy of an anonymous Abreview

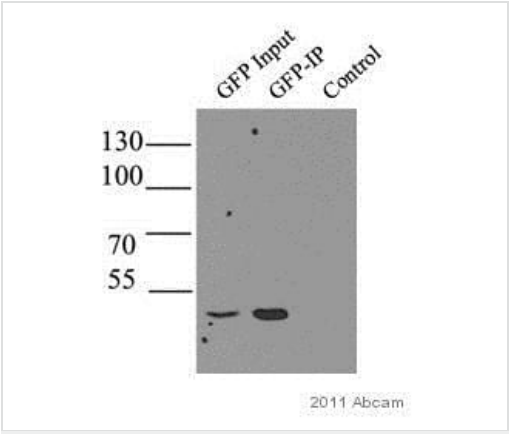
ab290 staining dog hearts (Adv-GFP injection) tissue sections by IHC-P. Sections were PFA fixed and subjected to heat mediated antigen retrieval in citric acid (Ph6.0, 0.05% Tween20) prior to blocking with 10% serum for 30 mins at 37°C. The primary antibody was diluted 1/1000 in PBS and incubated with the sample for 1 hour at 25°C. A HRP conjugated secondary like **Goat Anti-Rabbit IgG H&L (HRP) (ab205718)** was used.



Immunocytochemistry - Anti-GFP antibody (ab290)

Immunofluorescence images showing similar localization of Yes-GFP (first 10 aa's of Yes PTK fused to the N-terminus of GFP) to full length Yes PTK. A: Distribution of Yes detected using mouse anti-Yes Ab followed by Texas Red-conjugated anti-mouse Ab. B: Chimeric GFP's detected using rabbit anti-GFP Ab (Abcam ab290) followed by FITC-conjugated anti-rabbit Ab.

Image kindly provided by L.G. Berthiaume. Taken from J. McCabe and L.G. Berthiaume, Functional Roles for Fatty Acylated Amino-terminal Domains in Subcellular Localization, *Molecular Biology of the Cell* **10**:3771-3786, 1999

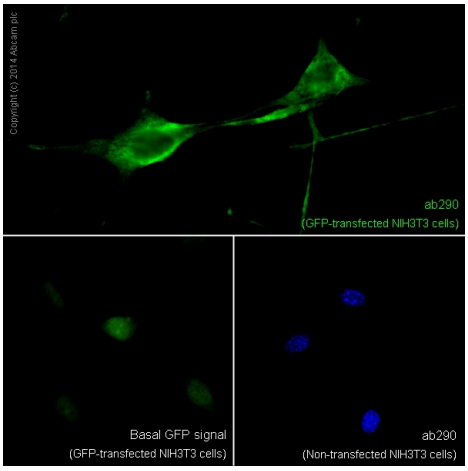


Immunoprecipitation - Anti-GFP antibody (ab290)

This image is courtesy of an Abreview submitted by William Hung

ab290 immunoprecipitating GFP in HEK293 nuclear lysate expressing GFP. 20µg of lysate was incubated with primary antibody (1 µg/mg lysate) and matrix (Protein G) for 16 hours at 4°C in AFC low salt buffer. For western blotting ab290 (1/5000) was used to confirm successful immunoprecipation.

Lane 1: HEK293 nuclear lysate expressing GFP input.
Lane 2: IP of HEK293 nuclear lysate expressing GFP.
Lane 3: Cells with no GFP.

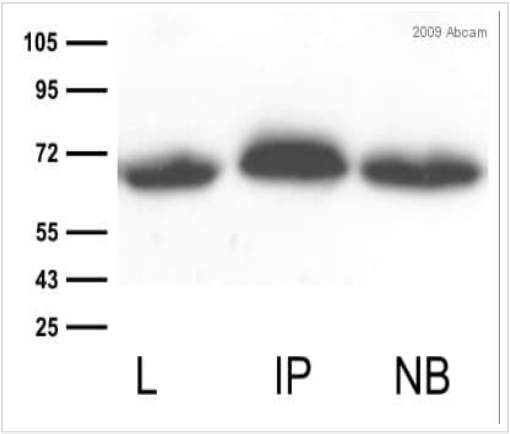


Immunocytochemistry - Anti-GFP antibody (ab290)

ab290 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 ab290 at 1/200 dilution overnight at +4°C followed by incubation with **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081)**, 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 ab290 was applied gave a stronger signal in the 488 channel, indicating that ab290 is binding to GFP and therefore eliciting signal amplification.

ab290 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).

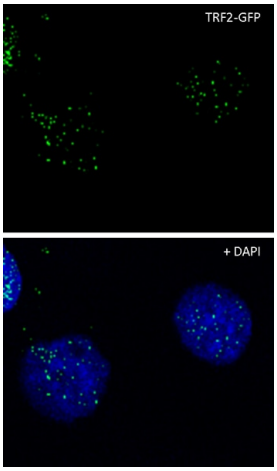


Immunoprecipitation - Anti-GFP antibody (ab290)

This image is courtesy of an Abreview submitted by Vladimir Milenkovic

ab290 immunoprecipitate in human HEK293 cells transfected with Annexin1-GFP. 25µg of cell lysate was incubated with the primary antibody and matrix (Protein G) in 1% TX-100, 10% glycerol, 1X PBS for 16 hours at 4°C. For Western blotting anti-rabbit HRP conjugated secondary antibody was used at a dilution at 1/5000.

Lane 1: Lysate of HEK293 cells expressing Annexin1-GFP fusion protein.
Lane 2: IP with anti-GFP.
Lane 3: Not bound fraction.

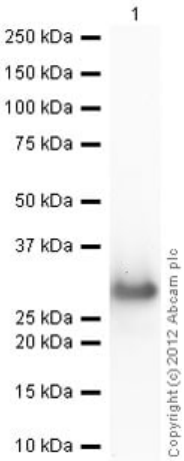


Immunocytochemistry - Anti-GFP antibody (ab290)

This image is courtesy of an anonymous Abreview

ab290 staining GFP in U2OS cells expressing TRF2-GFP fusion protein by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde, permeabilized with NP40 and blocked with 3% BSA for 1 hour at 21°C. Samples were incubated with the primary antibody (1/1000 in PBS + 3% BSA) for 12 hours at 4°C. An **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)** at a dilution of 1/500 was used as the secondary antibody.

Green - GFP.
Blue - DAPI.



Western blot - Anti-GFP antibody (ab290)

Anti-GFP antibody (ab290) at 1/2500 dilution + Recombinant A. victoria GFP protein (**ab84191**) at 0.01 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

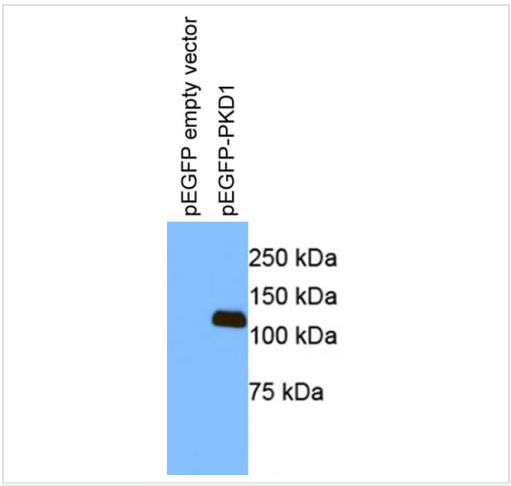
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 27 kDa

Exposure time: 30 seconds

Secondary antibody - goat **anti-rabbit HRP preadsorbed** (**ab97080**)



Western blot - Anti-GFP antibody (ab290)

This image is courtesy of an anonymous Abreview

All lanes : Anti-GFP antibody (ab290) at 1/5000 dilution

Lane 1 : LNCaP whole cell lysate - pEGFP empty vector
Lane 2 : LNCaP whole cell lysate - pEGFP-PKD1 transfected

Lysates/proteins at 20 µg per lane.

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

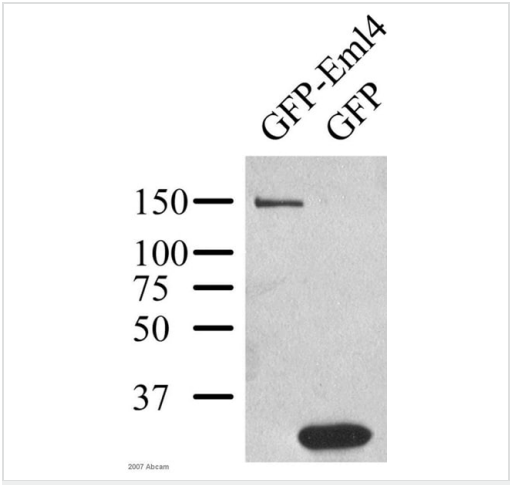
Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 10 seconds

Blocked with 5% milk for 1 hour at 23°C.

Incubated with the primary antibody for 16 hours at 4°C.



Western blot - Anti-GFP antibody (ab290)

This image is courtesy of an Abreview submitted by S Houtman

All lanes : Anti-GFP antibody (ab290) at 1/5000 dilution

Lane 1 : COS7 whole cell lysate - transfected with GFP-Eml4
Lane 2 : COS7 whole cell lysate - transfected with GFP

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP-conjugated pig anti-rabbit IgG at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 30 kDa

Exposure time: 10 seconds

Blocked with 5% milk for 1 hour at 20°C.

Incubated with the primary antibody for 18 hours at 4°C in TBS containing 2% milk and 1% Tween.

Predicted MW of Eml4 ~ 120 kDa.

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