# abcam

## Product datasheet

## Anti-GFP antibody ab5450

★★★★★ 13 Abreviews 210 References 5 图像

概述

产品名称 Anti-GFP抗体

描述 山羊多克隆抗体to GFP

宿主 Goat

经测试应用 适用于: IP, IHC-FoFr, Electron Microscopy, IHC - Wholemount, ICC/IF, WB, IHC-P

种属反应性 与反应: Species independent

免疫原 Recombinant full length protein corresponding to GFP.

Database link: P42212

**阳性**对照 Pure GFP protein, or cells known to overexpress GFP.

常规说明 Protein A will not bind goat lgG, so use alternates (eg. protein G) in IP with this antibody.

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

性能

形式 Liquid

**存放说明** 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.

**存储溶液** pH: 7.40

Constituents: 0.79% Tris HCI, 25% Glycerol

纯**度** Protein G purified

纯**化说明** This antibody is Goat anti-GFP serum (<u>ab5449</u>) affinity purified using a HiTrap-NHS activated

sepharose column (Amersham-Pharmacia) containing covalently linked highly purified

recombinant GFP. After applying the serum to the column and extensive washing, the eluted anti-

GFP immunoglobulins are desalted.

**克隆** 多克隆

**同种型** IgG

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#### The Abpromise quarantee

#### Abpromise™承诺保证使用ab5450于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IP	**** <u>(1)</u>	Use at an assay dependent concentration.
IHC-FoFr		Use at an assay dependent concentration.
Electron Microscopy		Use at an assay dependent concentration.
IHC - Wholemount	<b>★★★★★</b> (2)	Use at an assay dependent concentration.
ICC/IF	<b>★★★★★</b> (2)	1/2000.
WB		1/2000 - 1/20000.
IHC-P	<b>★★★★★</b> (4)	1/1000.

#### 靶标

### 相关性

**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<sup>2+</sup> -activated photoprotein aequorin.

Subunit structure: Monomer.

Tissue specificity: Photocytes.

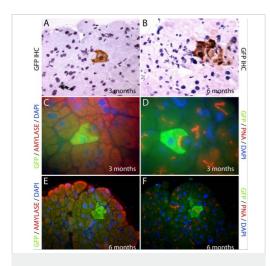
**Post-translational modification:** Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Ser-65 and Gly-67, and oxidation of Tyr-66 to didehydrotyrosine. Maturation of the chromophore requires nothing other than molecular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

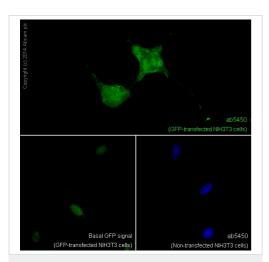
#### 图片



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody (ab5450)

Image from Sylva Met al., Plos One. 2011;6(10):e26088. Fig 2.; doi: 10.1371/journal.pone.0022616 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

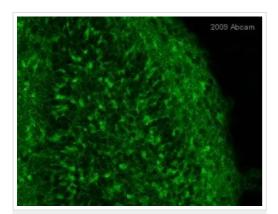
Immunohistochemical staining of donor BMDCs expressing GFP in mouse pancreas tissue using ab5450. Antigen retrieval was carried out using target-retrieval solution for 30 minutes in a boiling water bath. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide (5 minutes). Panels A and B were detected with a polymer horseradish peroxidase anti-rabbit detection system (30 minutes) and 3,3'-diaminobenzidine was used as a substrate. Counter-staining was performed with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody (ab5450) ab5450 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 ab5450 at 1/2000 dilution overnight at +4°C followed by incubation with <u>ab150129</u>, Donkey Anti-Goat IgG H&L (Alexa Fluor® 488), for 1 hour, at 1µg/ml.

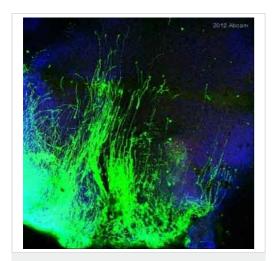
Under identical experimental conditions, when compared to the basal level of GFP expression in transfected NIH3T3 cells, the cells upon which ab5450 was applied gave a stronger signal in the 488 channel, indicating that ab5450 is binding to GFP and therefore eliciting signal amplification.

ab5450 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).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody (ab5450)
Image courtesy of an anonymous Abreview.

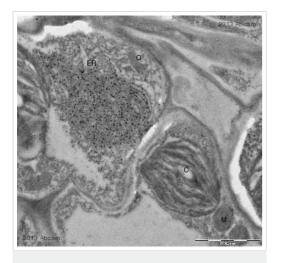
ab5450 staining GFP in murine brain tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with paraformaldehyde, permeabilized using 0.2% Triton and then blocked with serum for 1 hour at room temperature, followed by incubation with the primary antibody at a 1/1000 dilution for 14 hours at 4°C. A Cy2®-conjugated secondary antibody was used at a 1/200 dilution.



IHC - Wholemount - Anti-GFP antibody (ab5450)
Image courtesy of Sana Zakaria by Abreview.

ab5450 staining GFP in murine hindbrain tissue by IHC-Wholemount.

Dissected hindbrains of E12.5 stage embryos were fixed in 4% paraformaldehyde for 4-5 hours and then washed in PBS before proceeding with immunolabelling in PBS with 1% triton. Primary antibody was incubated for 4-6 days (99-144 hours). The hindbrain was cultured as an explant after GFP was electroporated. The wholemount immuno with ab5450 at a 1/500 dilution was done to see if GFP could be detected, it was the same GFP expression with the antibody as that which had been seen initially with the electroporation. The secondary used was an Alexa-Fluor 647 conjugated donkey anti-goat polyclonal used at a 1/150 dilution. Blue is DAPI and green is the GFP antibody (pseudo-colored from red Alexa Fluor 647).



Electron Microscopy - Anti-GFP antibody (ab5450)

This image is courtesy of an Abreview submitted by Narciso Campos

Electron Microscopy of Arabidopsis thaliana tissue sections labelling GFP with ab5450. An 18nm gold-conjugated Donkey anti-goat IgG polyclonal (1/15) was used as the secondary antibody.

Arabidopsis thaliana transgenic plant expressing GFP fused to an Endoplasmic Recticulum (ER) marker. No label is observed in: C - chloroplasts, M - mitochondria, G - golgi. The sample was prepared by cryofixation and embedding in Lowicryl HM20 resin. The image was taken in a JEOL 1010 transmission electron microscope at 20,000× magnification.

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