abcam

Product datasheet

Anti-GFP antibody [EPR14104] ab183734

Recombinant RabMAb

★★★★★ 23 Abreviews 76 References 11 Images

Overview

Product name Anti-GFP antibody [EPR14104]

Rabbit monoclonal [EPR14104] to GFP **Description**

Host species Rahhit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Species independent

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: GFP transfected 293 cell lysate. IHC-P: GFP transgenic mouse colon, pancreas and liver

tissue. Flow Cyt (intra): GFP transfected 293 cells. ICC/IF: GFP transfected NIH3T3 and 293 cells.

General notes On the basis of low sequence homology, ab183734 is predicted to show no or limited cross-

reactivity to RFP and BFP.

For the best results in IHC, please use ab183734 on Formalin/PFA-fixed paraffin-embedded

sections with heat mediated antigen retrieval.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our $\mathsf{RabMAb}^{\texttt{®}}$ technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

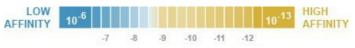
Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

 $K_D = 1.11 \times 10^{-11} M$ Dissociation constant (K_D)



Learn more about K_D

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR14104

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab183734 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 (Intra)		1/50.
WB	****(6)	1/10000 - 1/50000. Detects a band of approximately 27 kDa (predicted molecular weight: 27 kDa).
IHC-P	★★★★★ (7)	1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF	★★★★★ (8)	1/500.

Application notes

Is unsuitable for IP.

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 Ca²⁺ -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.

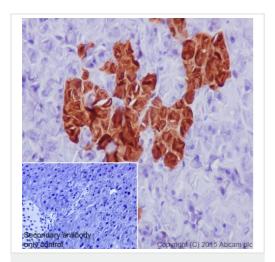
Biotechnological use: Green fluorescent protein has been engineered to produce a vast number of variously colored mutants, fusion proteins, and biosensors. Fluorescent proteins and its

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.

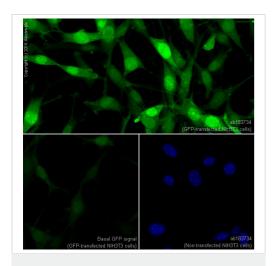
Biophysicochemical properties: Absorption: Abs(max)=395 nm Exhibits a smaller absorbance peak at 470 nm. The fluorescence emission spectrum peaks at 509 nm with a shoulder at 540 nm.

Images

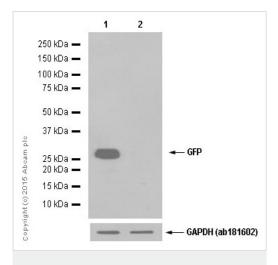


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody
[EPR14104] (ab183734)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of GFP transgenic mouse pancreas tissue labelling GFP with purified ab183734 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [EPR14104] (ab183734)



Western blot - Anti-GFP antibody [EPR14104] (ab183734)

ab183734 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 ab183734 at 1/500 dilution overnight at +4°C followed by incubation with $\underline{ab150081}$, Goat Anti-Rabbit lgG 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 ab183734 was applied gave a stronger signal in the 488 channel, indicating that ab183734 is binding to GFP and therefore eliciting signal amplification.

ab183734 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).

All lanes : Anti-GFP antibody [EPR14104] (ab183734) at 1/10000 dilution (purified)

Lane 1 : GFP transfected 293 cell lysate
Lane 2 : Non-transfected 293 cell lysate

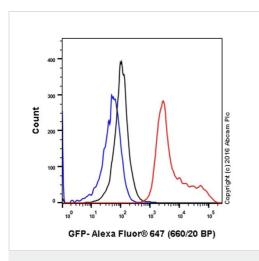
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

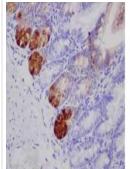
Predicted band size: 27 kDa **Observed band size:** 25 kDa

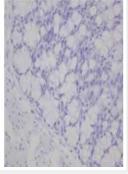
Blocking and dilution buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-GFP antibody [EPR14104] (ab183734)

Intracellular Flow Cytometry analysis of 293T (human embryonic kidney) transfected with human TNFRSF9 cells labeling GFP with purified ab183734 at 1/50 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor® 647) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

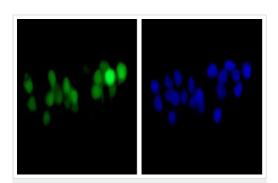




Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody
[EPR14104] (ab183734)

Immunohistochemical analysis of paraffin-embedded GFP transgenic mouse colon tissue (left) and normal mouse colon tissue (right) labeling GFP with unpurified ab183734 at 1/250 dilution followed by prediluted HRP Polymer for Rabbit lgG. Counterstained with Hematoxylin.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



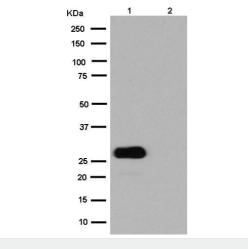
Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [EPR14104] (ab183734)

Immunofluorescent analysis of 4% paraformaldehyde-fixed GFP transfected 293 cells labeling GFP with unpurified ab183734 at 1/500 dilution, followed by Goat anti rabbit lgG (Alexa Fluor[®] 488) secondary antibody at 1/200 dilution (green). Counterstained with Dapi (blue)



Western blot - Anti-GFP antibody [EPR14104] (ab183734)

Different batches of ab183734 were tested on GFP transfected HEK-293 (Human embryonic kidney epithelial cell) lysate at 0.004 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 27 kDa.



Western blot - Anti-GFP antibody [EPR14104] (ab183734)

All lanes : Anti-GFP antibody [EPR14104] (ab183734) at 1/10000 dilution (unpurified)

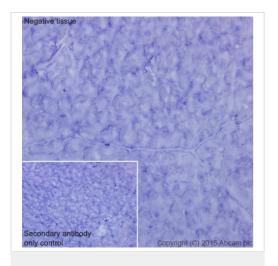
Lane 1 : GFP transfected 293 cell lysate
Lane 2 : Non-transfected 293 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

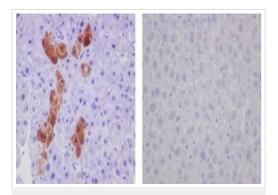
All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugate at 1/1000 dilution

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody
[EPR14104] (ab183734)

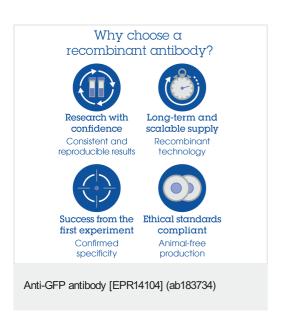
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse pancreas tissue (negative control) labelling GFP with purified ab183734 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFP antibody
[EPR14104] (ab183734)

Immunohistochemical analysis of paraffin-embedded GFP transgenic mouse liver tissue (left) and normal mouse liver tissue (right) labeling GFP with unpurified ab183734 at 1/250 dilution followed by prediluted HRP Polymer for Rabbit lgG. Counterstained with Hematoxylin.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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