abcam

Product datasheet

Anti-GFP antibody [EPR14104] - BSA and Azide free ab220802

Recombinant RabMAb

9 Images

Overview

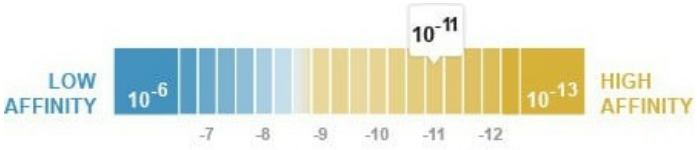
Product name	Anti-GFP antibody [EPR14104] - BSA and Azide free
Description	Rabbit monoclonal [EPR14104] to GFP - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Species independent
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 tissue and GFP transgenic mouse liver tissue. ICC/IF: GFP transfected 293 cells. ICC-IF: GFP transfected NIH3T3 cells.
General notes	ab220802 is the carrier-free version of **ab183734**. On the basis of low sequence homology, **ab183734** is predicted to show no or limited cross-reactivity to RFP, YFP, BFP, and CFP. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 RabMAb® 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. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 1.11 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR14104
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220802 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)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 27 kDa (predicted molecular weight: 27 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

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 Ca^{2+} -activated photoprotein aequorin.

Subunit structure: Monomer.

Tissue specificity: Photocytes.

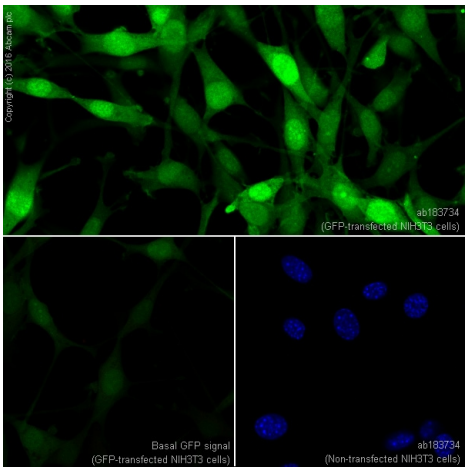
Post-translational modification: Contains a chromophore consisting of modified amino acid residues. The chromophore is formed by autocatalytic backbone condensation between Ser-65 and Gly-67, and oxidation of Tyr-66 to didehydrotyrosine. Maturation of the chromophore requires nothing other than molecular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



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

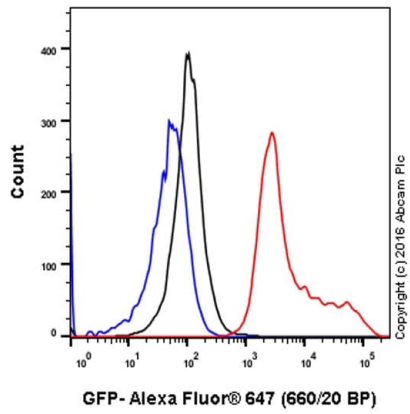
ab183734 staining GFP in GFP-transfected NIH3T3 cells. The cells were fixed with 4% formaldehyde (10min) and then blocked in 1% BSA / 0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with **ab183734** at 1/500 dilution overnight at +4°C followed by incubation with **ab150081**, Goat Anti-Rabbit 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 **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).

This data was developed using the same antibody clone in a

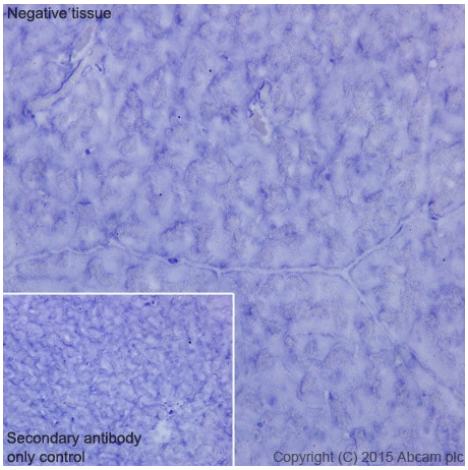
different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).



Flow Cytometry (Intracellular) - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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 IgG (Alexa Fluor® 647) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

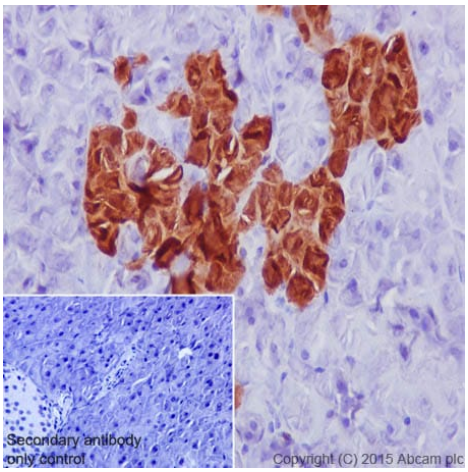
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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.

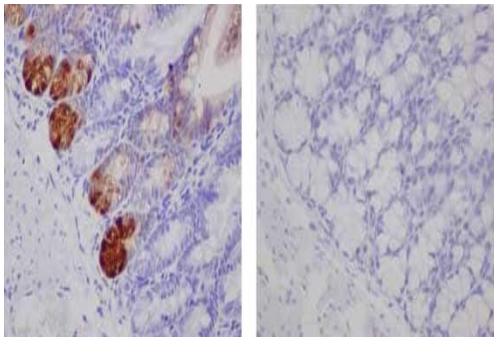
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).

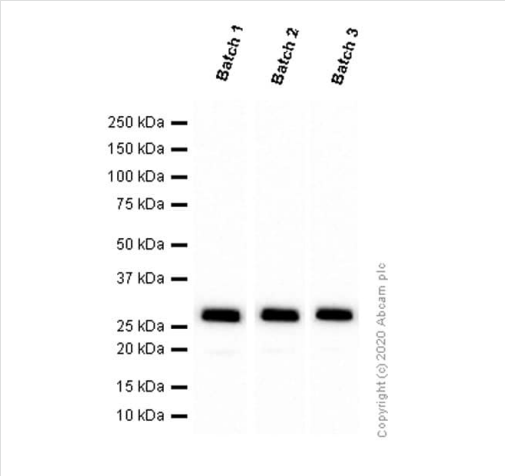


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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 IgG. Counterstained with Hematoxylin.

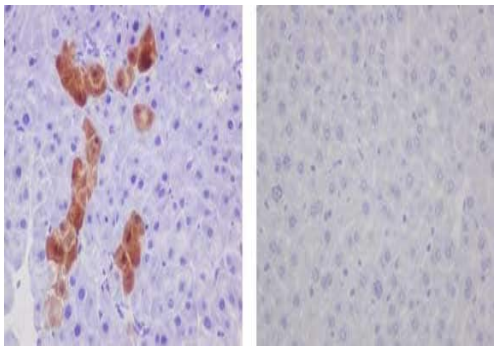
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).

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



Western blot - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

This data was developed using **ab183734**, the same antibody clone in a different buffer formulation. Different batches of **ab183734** were tested on GFP transfected HEK-293 (Human embryonic kidney epithelial cell) lysate at 0.004 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 27 kDa.

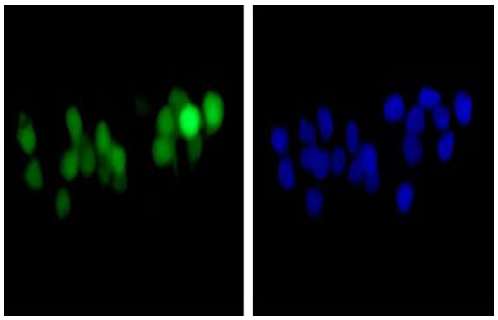


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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 IgG. Counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).

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



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183734**).

Anti-GFP antibody [EPR14104] - BSA and Azide free (ab220802)

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