abcam

Product datasheet

Anti-GFP antibody [3H9] ab252881

Recombinant

3 References 8 Images

Overview

Product name	Anti-GFP antibody [3H9]
Description	Rat monoclonal [3H9] to GFP
Host species	Rat
Specificity	This mAb recognizes eGFP, wild-type GFP, YFP and CFP.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Species independent
Immunogen	Full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T transfected with GFP expression vector, whole cell lysate. IHC-P: HEK-293T transfected with a GFP construct. ICC/IF: HEK-293T cells.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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**.

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.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	3H9

Isotype IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab252881 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		1/5000. Predicted molecular weight: 27 kDa.
IHC-P		1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins
ICC/IF		1/100.

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.

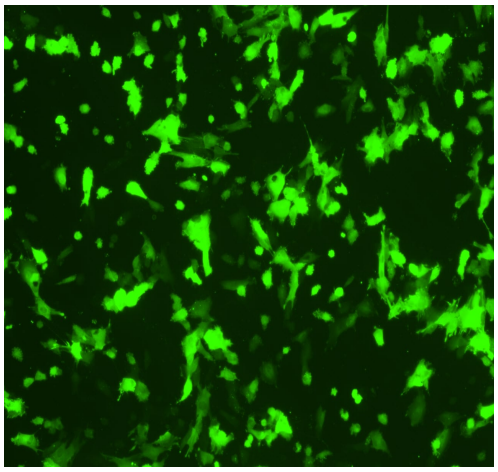
Biotechnological use: Green fluorescent protein has been engineered to produce a vast number of variously colored mutants, fusion proteins, and biosensors. Fluorescent proteins and its mutated allelic forms, blue, cyan and yellow have become a useful and ubiquitous tool for making chimeric proteins,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

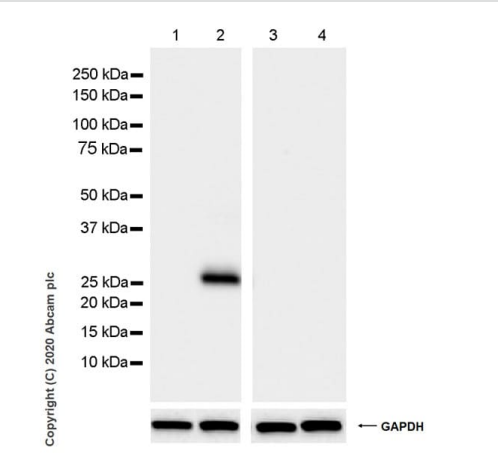
Images



Immunocytochemistry - Anti-GFP antibody [3H9] (ab252881)

This image is courtesy of an Abreview submitted by Prashant Rai

Immunocytochemistry analysis of paraformaldehyde-fixed 1x PBS permeabilized porcine fetal fibroblast cells staining with ab252881. Secondary antibody was Alexa Fluor™ 594 IgG(H+L). Samples were incubated with the primary antibody with PBS and BSA for 12 hours at 4°C. Blocking was done using 1% BSA for 1 hour at 25°C.



Western blot - Anti-GFP antibody [3H9] (ab252881)

All lanes : Anti-GFP antibody [3H9] (ab252881) at 1/5000 dilution

Lane 1 : HEK-293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 2 : HEK-293T transfected with GFP expression vector, whole cell lysate
Lane 3 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 4 : C6 (rat glial tumor glial cell), whole cell lysate

Lysates/proteins at 10 µg per lane.

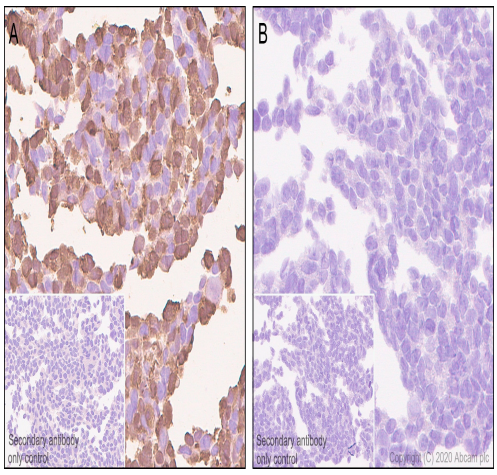
Secondary
All lanes : Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/100000 dilution

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

Exposure time: 3 seconds

Blocking and Diluting buffer: 5% NFDM/TBST

Exposure time: 3 seconds

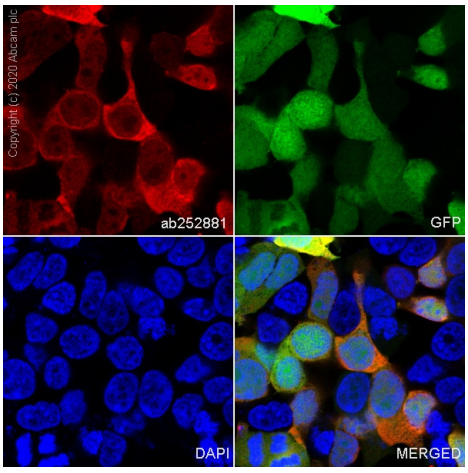


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [3H9] (ab252881)

Immunohistochemical analysis of paraffin-embedded HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with a GFP construct (A) labeling GFP with ab252881 at 1/2000 dilution (0.539 µg/ml) followed by a ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**). No staining observed on HEK-293T cells (B). The section was incubated with ab252881 for 30 mins at room temperature. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**).

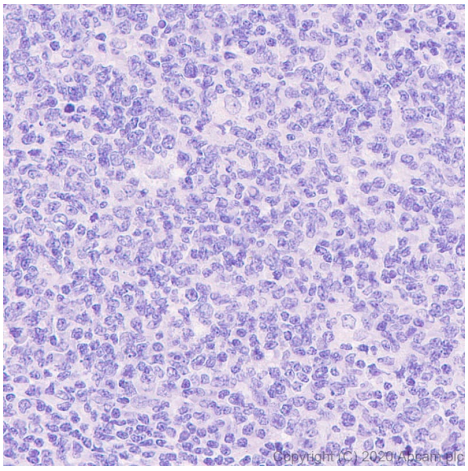
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody [3H9] (ab252881)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells stained for GFP (red) using ab252881 at 1/100 dilution (10.78 µg/ml), followed by **ab150160** Goat Anti-Rat IgG H&L (Alexa Fluor® 594) antibody at 1/1000 dilution. Confocal image showing cytoplasmic and nuclear staining in HEK-293T cells transfected with GFP only plasmid. The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150160** Goat Anti-Rat IgG H&L (Alexa Fluor® 594) at 1/1000 dilution.



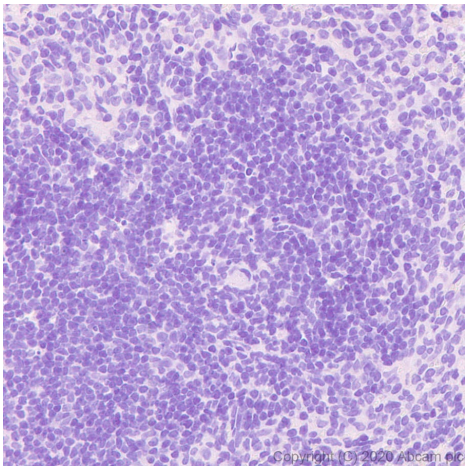
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [3H9] (ab252881)

Immunohistochemical analysis of paraffin-embedded human tonsil section labeling GFP with ab252881 at 1/2000 dilution (0.539 µg/ml) followed by a ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**). The section was incubated with ab252881 for 30 mins at room temperature. Counterstained with Hematoxylin.

Negative control: no staining on human tonsil.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



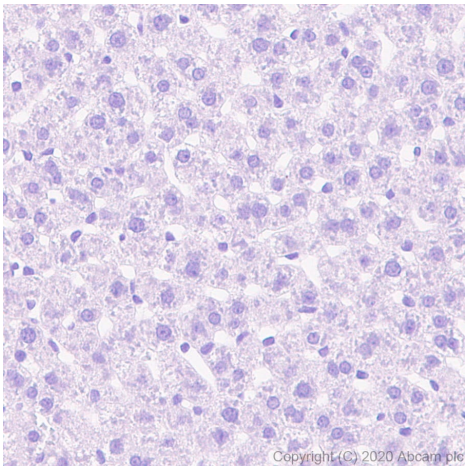
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [3H9] (ab252881)

Immunohistochemical analysis of paraffin-embedded mouse spleen section labeling GFP with ab252881 at 1/2000 dilution (0.539 µg/ml) followed by a ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**). The section was incubated with ab252881 for 30 mins at room temperature. Counterstained with Hematoxylin.

Negative control: no staining on mouse spleen.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



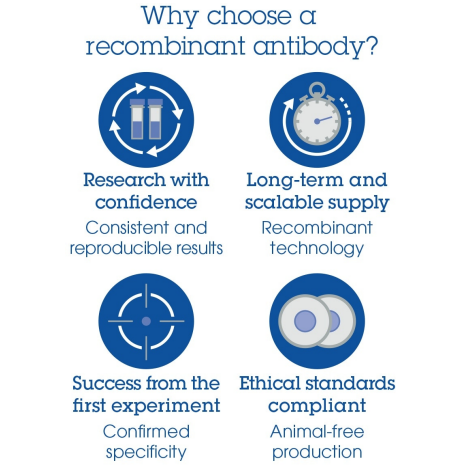
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody [3H9] (ab252881)

Immunohistochemical analysis of paraffin-embedded rat liver section labeling GFP with ab252881 at 1/2000 dilution (0.539 µg/ml) followed by a ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**). The section was incubated with ab252881 for 30 mins at room temperature. Counterstained with Hematoxylin.

Negative control: no staining on rat liver.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-rat IgG H&L (HRP polymer) (**ab214882**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Anti-GFP antibody [3H9] (ab252881)

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