Overview

Product name: Anti-GFP antibody

Description: Rabbit polyclonal to GFP

Host species: Rabbit

Specificity: This 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: IHC-FoFr, Electron Microscopy, IHC-P, IP, WB, ICC/IF, Flow Cyt

Immunogen: Recombinant full length protein corresponding to GFP.

Database link: P42212

Positive control: Pure GFP protein, or cells known to overexpress GFP can be used as a positive control in WB.

General notes: Please note that a mistake was made in reference 4 (Mesaeli et.al., J. Cell. Biol. 1999 Mar 8;144(5):857-68). The antibody used for immunohistochemistry on paraformaldehyde fixed tissues was the crude serum version of this antibody (Abcam ab290) and not Clontech’s monoclonal as stated. This product is supplied in 25% glycerol. During freezing and thawing some phase separation might occur - Please ensure that the solution is mixed thoroughly but GENTLY before use.

This antibody (ab6556) is the purified version of our best-selling rabbit polyclonal to GFP (ab290). It has been developed specifically for use in applications requiring a high titre and specificity with minimum background such as immuno-electron microscopy.

This anti-GFP antibody recognizes the enhanced form of GFP as well.

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

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: pH: 7.40

Constituents: 0.79% Tris HCl, 25% Glycerol

Purity: Immunogen affinity purified
Purification notes
This antibody is an affinity purified rabbit anti-GFP antibody purified on an affinity chromatography column made with highly purified recombinant GFP.

Clonality
Polyclonal

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab6556 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-FoFr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 19563657</td>
</tr>
<tr>
<td>Electron Microscopy</td>
<td></td>
<td>1/5000.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. Use Protein A agarose for IP.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000 - 1/5000.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
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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.

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**Images**

Regionally restricted CX3CL1 expression in the retina of adult CX3CL1 cherry: CX3CR1gfp mouse (A-E)

Fluorescent light microscopy analysis of a vertical retina cryosection (A) showing both CX3CL1/Cherry (detected in rhodamine channel, red) (B) and CX3CR1/GFP (detected in FITC channel, green) (C) reporters expression. CX3CR1/GFP signal is assigned to microglial cells with cell bodies and dendritic ramifications in three sublayers (outer plexiform layer, OPL, inner plexiform layer, IPL and ganglion cells layer, GCL). CX3CL1/Cherry fluorescent cells are found exclusively in ganglion cell and inner nuclear layer. No fluorescent cells were observed in outer nuclear layer (ONL). Enlargements show strong Cherry signal in a presumable amacrine cell body in inner nuclear (D, arrow points to dendritic base) and ganglion cell (E) layers, indicating high level of the CX3CL1 chemokine promotor activity in neurons. Cryosections are counterstained with DAPI nucleic acid stain (blue). Distribution of the Cherry reporter positive cells in the retina of CX3CL1cherry: CX3CR1gfp mice coincides mostly with results obtained in *in situ* hybridization studies. Of note, membrane/lipid inner and outer photoreceptor segment layer exhibit nonspecific fluorescence. Scale bars represent 50 µm (A, B and C), and 10 µm (D, E).

GFP was detected with ab6556 at 1/1000 dilution.

(From Figure 4 of Zieger et al)
The downstream Wnt non-canonical pathway components are required in the escort cells for cystoblast encapsulation

(A-D1) Germaria of c587-GAL4, RhoA, Rac1 and cdc42 depleted escort cells stained with Tj (red), GFP (green) and Vasa (blue) showing loss of encapsulation in RhoA, Rac1 and cdc42 depleted escort cells. Fax-GFP marks somatic cell membranes. GFP channel is shown in A1, B1, C1 and D1. Scale bar for all images is 20μm.

GFP is detected with ab6556.
(After Figure 2 of Upadhyay et al)

All lanes: Anti-GFP antibody (ab6556) at 1/5000 dilution

Lane 1: LNCaP cells pEGFP-PKD1 empty vector
Lane 2: LNCaP pEGFP-PKD1 transfected cells

Secondary

All lanes: Rabbit polyclonal to GFP (ab6556) at 1/10000 dilution (Goat anti-rabbit HRP)

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 10 seconds

ab6556 staining GFP in mouse tooth tissue section by Immunohistochemistry (Frozen sections) without fixation. Tissue samples were blocked with 1% BSA for 20 minutes at 20°C. The sample was incubated with primary antibody (1/500) for 2 hours at 20°C. An Alexa Fluor®488-conjugated Chicken polyclonal to rabbit IgG was used as secondary antibody at 1/200 dilution. Immunofluorescent localization of CD31 and GFP in implanted non GFP cultured molars in GFP adult mice showed the origin of neo-formed blood vessels.

DP: Dental Pulp
EO: Enamel Organ
This image shows a single primary hippocampal neuron from a primary culture overexpressing GFP stained with ab6556 at a dilution of 1/2000. This picture was kindly supplied as part of the review submitted by one of our customers.

This image shows IF using GFP-expressing glial cells (green) transplanted into lesioned rat spinal cord. This was detected using ab6556 anti-GFP antibody and a FITC conjugated secondary antibody. Axons are labelled red by an antibody to neurofilament-200 and a rhodamine secondary. ab6556 reveals the morphology of the transplanted cells to such an extent that their close interactions with axons are obvious. The top picture shows an optical section from a confocal microscope scan showing how a GFP cell wraps around a branched axon travelling longitudinally. The bottom picture consists of an optical section from another confocal scan showing a GFP cell enveloping an axon in the transverse plane. Review by Andrew Toft submitted 19 May 2004.

ab6556 at 1/500 dilution staining GFP in mouse testis by immunohistochemistry (frozen sections). Sections were paraformaldehyde fixed prior to blocking in 100% serum for 1 hour at 37°C and then incubated with ab6556 for 1 hour at 37°C. A Texas Red conjugated chicken polyclonal to rabbit Ig, diluted 1/100, was used as the secondary antibody.
Specific labeling of a Trk-GFP fusion protein being synthesized on ER in sympathetic neurons infected with an adenovirus carrying the construct. The gold is associated with the ER membranes. This was done using a 1/5000 dilution of affinity purified antibody (ab6556). The tissue section was fixed and embedded using durcupan resin.

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