Product datasheet

Anti-GFP antibody (FITC) ab6662

Overview

Product name
Anti-GFP antibody (FITC)

Description
Goat polyclonal to GFP (FITC)

Host species
Goat

Conjugation
FITC. Ex: 493nm, Em: 528nm

Tested applications
Suitable for: IHC-FoFr, IHC-P, IHC-Fr, WB, ICC/IF

Immunogen
Recombinant full length protein corresponding to GFP aa 1-246.
Sequence:
MSKGEELFTGVPIVLDGVDJYKFSVSGEKEGDAFLTGKLTKFICTT
GKLVPVPWPTL
VTFSYGVQCFSRYPDHMKQHDDFAMPEQYQERTIFKDDGNYKTRA
EVKFEGDLTV
NRELKIDFKEDGKLEYNSSNVYIMADKQNGIKVNFIRH
IEdGSVQAD
HYQNTPIGDGPVLLLDPNHYLSTQSALSKGDPNKRDMVLEFVTAGIT
HGMDELYK

Database link: P42212

General notes
Designed to detect GFP and its variants in ELISA (sandwich or capture), immunoblotting and immunoprecipitation. Fluorescein conjugated anti-GFP was assayed by immunofluorescence microscopy on prokaryotic (E.coli) and eukaryotic (CHO cells) expression systems and was shown to detect GFP containing inserts. Significant amplification of signal was detected using fluorochrome conjugated anti-GFP relative to the fluorescence of GFP alone.

In case of unexpected background, use pre-adsorbed secondary antibodies.

Fluorescein isothiocyanate (FITC) (MW 390 daltons) Absorption Wavelength: 495 nm Emission Wavelength: 528 nm Fluorochrome/Protein Ratio: 3.5 moles FITC per mole of Goat IgG

Properties

Form
Liquid

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 0.42% Potassium phosphate, 0.87% Sodium chloride, 1% BSA

BSA Immunoglobulin and Protease free

**Purity**
Protein G purified

**Purification notes**
GFP Fluorescein Conjugated Antibody was prepared from monospecific antiserum by immunoaffinity chromatography using Green Fluorescent Protein (Aequorea victoria) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities.

**Primary antibody notes**
Designed to detect GFP and its variants in ELISA (sandwich or capture), immunoblotting and immunoprecipitation. Fluorescein conjugated anti-GFP was assayed by immunofluorescence microscopy on prokaryotic (E.coli) and eukaryotic (CHO cells) expression systems and was shown to detect GFP containing inserts. Significant amplification of signal was detected using fluorochrome conjugated anti-GFP relative to the fluorescence of GFP alone.

**Clonality**
Polyclonal

**Isotype**
IgG

**Applications**

Our [Abpromise guarantee](#) covers the use of [ab6662](#) in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-FoFr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/250.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/10000 - 1/100000.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200 - 1/400.</td>
</tr>
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</table>

**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

Immunofluorescence Microscopy using ab6658. Tissue: Drosophila melanogaster late stage embryonic central nervous system. Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: Anti-GFP antibody at a 1/1,000 for 1 h at RT. Secondary antibody: AlexaFluor 488™ conjugated anti-Goat antibody at 1/300 for 45 min at RT. Panel A: shows a lateral view (ventral left). Panels B and C: shows ventral views of whole mount embryos at 63x magnification (plus 2x digital zoom). In all panels, anterior is up. Staining: tau-GFP cell bodies (large arrowhead) and axons of motorneurons (arrow) and interneurons (small arrowhead) as green fluorescent signal.
Western blot - Anti-GFP antibody (FITC) (ab6662) + Recombinant A. victoria GFP protein (ab84191)

Western Blot of ab6662. Load: 50 ng per lane. Secondary antibody: Fluorescein goat secondary antibody at 1/1,000 for 60 min at RT. Block: MB-070 for 30 min at RT.

Immunohistochemistry (Frozen sections) - Anti-GFP antibody (FITC) (ab6662)

ab6662 staining mouse brain tissue sections (inducible GFP reporter) by IHC-Fr. The tissue was paraformaldehyde fixed and blocked with serum and then incubated with the antibody at a 1/1000 dilution for 1 hour.

Staining is shown in the left hand panel. The middle panel shows staining with a rabbit anti-GFP antibody and the right hand panel shows the merged images (plus DAPI). ab6662 gives no noticeable background and it is found that when viewing on an epifluorescent the exposure time is significantly reduced.
Immunofluorescence Microscopy using ab6658. Tissue: Sf-1:Cre mice crossed to the Z/EG reporter line. Mouse brain (coronal view, 20X magnification). Fixation: 4%PFA/PBS with o/n fixation, and subsequently transferred to a 30% sucrose solution. Antigen retrieval: frozen in OCT freezing medium (Sakura) and cryostat sectioned at 40 microns. Primary antibody: Goat anti-GFP was used at 1/500 dilution in free floating immunohistochemistry to detect GFP. Secondary antibody: Fluorochrome conjugated Anti-goat IgG secondary antibody was used for detection at 1:500 at 1/10,000 for 45 min at RT. Localization: Sf-1+ neurons and their processes of the ventromedial nucleus of the hypothalamus. Staining: eGFP as green fluorescent signal and sections were counterstained with DAPI.

These pictures show confocal immunofluorescence using GFP-expressing glial cells (green) transplanted into the lesioned rat spinal cord. This was detected using ab6662 and a standard FITC filter set. Axons are labelled red by an antibody to neurofilament-200 and a rhodamine secondary antibody. The upper panel shows the centre of the transplant site at low power. Numerous GFP-positive cells can be seen mingling with axons. The lower panel shows, at high power in a single optical section, how ab6662 reveals the morphology of the transplanted cells to such an extent that their close interactions with axons are obvious - the cell depicted can be seen wrapping around a neurofilament-200 positive axon.

These images were kindly supplied as part of the review submitted by Andrew Toft.

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