# abcam

### Product datasheet

# Sepharose Anti-GFP antibody ab69314

## ★★★★★ 1 Abreviews 27 References 1 Image

#### Overview

Product name Sepharose Anti-GFP antibody

**Description** Sepharose Rabbit polyclonal to GFP

Host species Rabbit

**Conjugation** Sepharose

**Specificity** This product is a suspension of affinity purified anti-GFP antibody (ab6556) covalently linked to

sepharose beads. It is reactive against all variants of *Aequorea victoria* GFP such as S65T-GFP, RS-GFP, YFP, CFP, RFP, and EGFP. The unit sold contains  $25\mu g$  of affinity purified rabbit anti-GFP lgG cross-linked to  $125 \mu l$  sepharose beads in a total volume of  $250 \mu l$  buffer. The product is supplied as a 50% slurry to facilitate pipetting. Pipetting the slurry is facilitated when pipet tips are

blunted by cutting 3-5mm off from the tip.

Tested applications Suitable for: 

□

Species reactivity Reacts with: Species independent

**Immunogen** Recombinant full length protein corresponding to GFP.

Database link: P42212

**Positive control** Pure GFP protein, or cells known to overexpress GFP.

General notes This product should be kept refrigerated at all times whilst in short term storage. Using sterilised

equipment will reduce the risk of bacterial contamination.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Storage buffer** Preservative: 0.05% Sodium azide

Constituent: PBS

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**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**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab69314 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
IP	★ ★ ★ ★ 👚 (1)	Use at an assay dependent concentration.  We rountinely recommend using 20 µL of slurry per IP, which corresponds to 2 µg of IgG cross-linked to beads. this results in a clearly visible 10 µL bead pellet upon centrifugation.  Unconjugated version: <a href="mailto:ab6556"><u>ab6556</u></a> . FITC conjugated version: <a href="mailto:ab69313"><u>ab66180</u></a> . Biotin conjugated version: <a href="mailto:ab69313"><u>ab69313</u></a> .

#### **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<sup>2+</sup> -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.

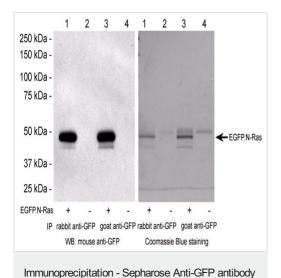
Sequence similarities: Belongs to the GFP family.

#### 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**

(ab69314)



Lane 1 & 3: IP from COS 7 cells transfected with EGFP.N-Ras (48 kDa)

Lane 2 & 4: IP from untransfected COS 7 cells

Lane 1 & 2: IP using 15 ul of rabbit anti-GFP conjugated to sepharose beads (0.5 mg lgG per ml of beads)

Lane 3 & 4: IP using 15 ul of goat anti-GFP conjugated to sepharose beads (1 mg lgG per ml of beads)

Immunoprecipitation: COS 7 cell lysates containing 100 ug of total protein in 200 ul of 0.1% SDS-RIPA buffer with addition of complete protease inhibitor were used for each immunoprecipitation. Cell lysates were incubated with anti-GFP sepharose beads for 2 hours at 4oC with rocking. Beads were washed. Proteins were eluted with 1% SDS 50 mM HEPES (pH 7.4) at 80oC (15 min). Half of the IP sample was loaded on each lane of 10 % SDS PAGE gel and gel was processed for Western blotting/ECL.

Western blot: Primary antibody: monoclonal mouse anti-GFP (ab291) at 0.2 ug/ml in 5% non-fat milk/TBS-T; 1 hour incubation at room

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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