Product name: Anti-GFP antibody

Description: Chicken polyclonal to GFP

Host species: Chicken

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

Immunogen: Recombinant full length protein corresponding to GFP.

Database link: P42212

Positive control: ICC/IF: GFP-transfected NIH3T3 cells

Form: Liquid


Storage buffer: Preservative: 0.01% Thimerosal (merthiolate)
Constituents: PBS, 50% Glycerol, 0.16% Sodium phosphate

Purity: IgY fraction

Purification notes: Sterile filtered.

Clonality: Polyclonal

Isotype: IgY

Applications:

Our Abpromise guarantee covers the use of ab13970 in the following tested applications.

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

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<td>IHC-P</td>
<td>★★★★☆☆</td>
<td>1/500 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The concentrations of fixative for the IHC applications were typically 10% formalin or 2% paraformaldehyde.</td>
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**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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**Application** | **Abreviews** | **Notes**
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**WB** |  | 1/5000.

**IHC - Wholemount** |  | Use at an assay dependent concentration.

**IHC-FrFI** |  | Use at an assay dependent concentration.

**ICC/IF** |  | 1/2000. Used at a dilution of 1/2000 for 1 hr (see Abreview for further information).

**IHC-Fr** |  | 1/1000.

**IHC-FoFr** |  | 1/2000.

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**Target**
ab13970 staining GFP in Human U2OS cells by ICC/IF. Cells were paraformaldehyde fixed, permeabilized with 0.5% triton and blocked with 2% antibody dilution buffer for 2 hours. Cells were incubated with the primary antibody (1/1000) for 1 hour at 25°C. An undiluted Alexa Fluor® 488 conjugated Goat anti-chicken polyclonal was used as the secondary antibody.
**Western blot analysis** of HEK293 transfected and untransfected cell lysates, labelling GFP with ab13970. Cells were treated by mixing in RIPA buffer and denaturating in Laemmli buffer for 5 mins at 95°C. The gel was Precast at 4-12%. Blocking was with 0.5% milk at 20°C for 5 mins.

**All lanes:** Anti-GFP antibody (ab13970) at 1/200 dilution

**Lane 1:** Wild type 'naive' HEK293 whole cell lysates

**Lanes 2-4:** GFP transfected HEK293 whole cell lysates

Lysates/proteins at 5 µg per lane.

**Secondary**

**All lanes:** Donkey anti-chicken polyclonal CY5 conjugate. at 1/1000 dilution

Performed under reducing conditions.

**Observed band size:** 25 kDa

**Exposure time:** 25 minutes
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFP antibody (ab13970)

Image courtesy of an anonymous Abreview.

ab13970 staining GFP in murine lung tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue was fixed in paraformaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer. Samples were then permeabilized with 0.1% Tween, blocked with 15% serum for 30 minutes at 23°C and then incubated with ab13970 at a 1/500 dilution for 14 hours at 4 °C. The secondary used was an Alexa-Fluor 488 conjugated goat anti-chicken polyclonal used at a 1/500 dilution.

Immunohistochemistry (Frozen sections) - Anti-GFP antibody (ab13970)

This image is courtesy of an anonymous Abreview.

ab13970 staining mouse olfactory bulb tissue sections by IHC-Fr. Sections were PFA fixed, permeabilized in 0.4% Triton-X and blocked with 5% serum for 2 hours at 25°C. The primary antibody was diluted 1/1000 and incubated with the sample for 16 hours at 4°C. An Alexa Fluor® 488 conjugated goat anti-chicken was used as the secondary.

Western blot - Anti-GFP antibody (ab13970)

Western blot of transgenic mouse spinal cords showing that the rabbit anti-GFP (lane 1) and the chicken anti-GFP (Abcam; lane 2) recognize a band at the same molecular weight.

Western blot of transgenic mouse spinal cords showing that the rabbit anti-GFP (lane 1) and the chicken anti-GFP (Abcam; lane 2) recognize a band at the same molecular weight.
Transgenic mice expressing GFP selectively in lamina II of the spinal cord. In the right panels, note the correspondence between the green (rabbit anti-GFP) and red signals (chicken anti-GFP from Abcam) indicating that these two antibody preparations recognized the same gene product. The secondary antibody used with ab13970 was a FITC-labeled goat anti-chicken

ab13970 staining GFP in GFP-transfected NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 min) and then blocked in 1% BSA / 0.3 M glycine in 0.1% PBS-Tween for 1 h. The cells were then incubated with ab13970 at 1/2000 dilution overnight at +4°C followed by incubation with Goat Anti-Chicken IgY H&L (Alexa Fluor® 488) preadsorbed (ab150173), for 1 hour, at 1 μg/ml.

Under identical experimental conditions, when compared to the basal level of GFP expression in transfected NIH3T3 cells, the cells upon which ab13970 was applied gave a stronger signal in the 488 channel, indicating that ab13970 is binding to GFP and therefore eliciting signal amplification.

ab13970 was also applied to non-GFP-transfected NIH3T3 cells, which produced no positive staining, indicating specificity for GFP. Nuclear DNA was labelled with 1.43 μM DAPI (blue).
**Immunohistochemistry (Frozen sections) - Anti-GFP antibody (ab13970)**

This image is courtesy of an Abreview submitted by Dr Schwob’s Lab

ab13970 staining mouse olfactory epithelium tissue sections by IHC-Fr. The sample was PFA fixed and blocked with 4% BSA/5% NFDM/10% NDS for 15 minutes at 20°C. The primary antibody was diluted 1/1000 and incubated with the sample for 1 hour. A FITC conjugated goat anti-chicken was used as the secondary.

This colony is the result of retroviral infection with a control virus. The GFP is under the control of an IRES promoter, so its expression is independent of any other protein. The counter-stain is hoescht.

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**Western blot - Anti-GFP antibody (ab13970)**

Image courtesy of an anonymous Abreview.

**All lanes**: Anti-GFP antibody (ab13970) at 1/1000 dilution

**All lanes**: Whole cell lysate prepared from HeLa cells.

Lysates/proteins at 25 µg per lane.

**Secondary**

**All lanes**: IRDye 800CW conjugated goat anti-chicken polyclonal at 1/15000 dilution

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**Immunocytochemistry/ Immunofluorescence - Anti-GFP antibody (ab13970)**

This image is courtesy of an Abreview submitted by Dr Radbod Darabi

ab13970 staining GFP + tumor in mouse muscle cells by ICC/IF. Cells were formaldehyde fixed and blocked with 3% BSA for 1 hour at 24°C prior to incubation with the primary antibody (1/500) for 1 hour at 24°C. An Alexa Fluor® 488 conjugated goat anti-chicken was used as the secondary.
Immunohistochemistry (Frozen sections) - Anti-GFP antibody (ab13970)
This image is a courtesy of Ben Deverman

ab13970 staining GFP in mouse brain tissue section by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed with paraformaldehyde and permeabilized with 0.1% Triton X-100 before blocking with 10% serum for 30 minutes at 25°C. The sample was incubated with primary antibody (1:2000) for 16 hours at 25°C in 10% NGS in PBS + 0.1% TX100. An Alexa Fluor®488-conjugated Goat polyclonal to chicken IgG was used as secondary antibody at 1:400 dilution. In the image, green staining represents GFP expressed in oligodendrocytes, blue is for ToPro3.

Immunohistochemistry (Frozen sections) - Anti-GFP antibody (ab13970)

ab13970 staining GFP in murine olfactory bulb tissue by Immunohistochemistry (PFA perfusion fixed frozen sections) Counterstained with DAPI.

Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-GFP antibody (ab13970)

Immunocytochemical immunofluorescence analysis of human cytospined HEK293 cells transfected with GFP, labelling GFP with ab13970 at 1/200 incubated for 16 hours at 4°C with 1% BSA in PBS. Secondary used was a donkey anti-chicken polyclonal DyLight® 594 at 1/500. GFP is shown in red (DyLight® 594). Nuclei are counterstained in blue (DAPI). The left pane shows HEK293 cells transfected with GFP and the right pane shows non-transfected HEK293 cells.

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