abcam

Product datasheet

Anti-EGFP antibody [F56-6A1.2.3] ab184601

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Overview

Product name	Anti-EGFP antibody [F56-6A1.2.3]
Description	Mouse monoclonal [F56-6A1.2.3] to EGFP
Host species	Mouse
Tested applications	**Suitable for:** WB, ICC/IF
Species reactivity	**Reacts with:** Species independent
Immunogen	Full length protein corresponding to EGFP aa 1 to the C-terminus. enhanced green fluorescent protein (eGFP). Native protein Database link: **CAD97424.1**
Positive control	ICC/IF: U-2 OS cells transfected with an EGFR-eGFP fusion protein. WB: Recombinant GFP.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, PBS
Purity	Protein A purified
Purification notes	in vitro produced
Clonality	Monoclonal
Clone number	F56-6A1.2.3
Isotype	IgG2b

Applications

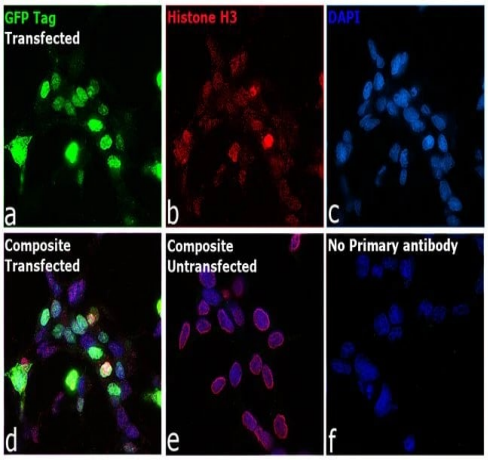
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab184601 in the following tested applications.

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

Application	Abreviews	Notes
WB	★★★★☆ **(1)**	1/1000. Predicted molecular weight: 27 kDa.
ICC/IF		1/20 - 1/100.

Target

Images

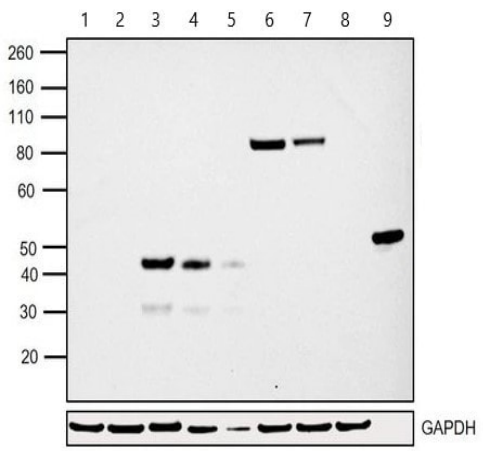


Immunocytochemistry/ Immunofluorescence - Anti-EGFP antibody [F56-6A1.2.3] (ab184601)

Immunofluorescent analysis of GFP Tag was performed using H3-GFP construct transfected in HEK-293E cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with ab184601 at 1/100 dilution and Histone H3 Rabbit Polyclonal Antibody at 0.5 µg/mL in 0.1% BSA, incubated at 4°C overnight and then labeled with Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® Plus 555 and Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor® Plus 647 respectively at a dilution of 1/2000 for 45 minutes at room temperature.

Image a: Anti-EGFP antibody [F56-6A1.2.3], ab184601 (green)
Image b: Histone H3 (red)
Image c: Nuclei stained with DAPI (blue)
Image d: Merged image showing the co-localization of nuclear signals in transfected cells
Image e: Untransfected HEK cells
Image f: Control cells with no primary antibody to assess background

The images were captured at 60X magnification.



Western blot - Anti-EGFP antibody [F56-6A1.2.3] (ab184601)

All lanes : Anti-EGFP antibody [F56-6A1.2.3] (ab184601) at 1/1000 dilution

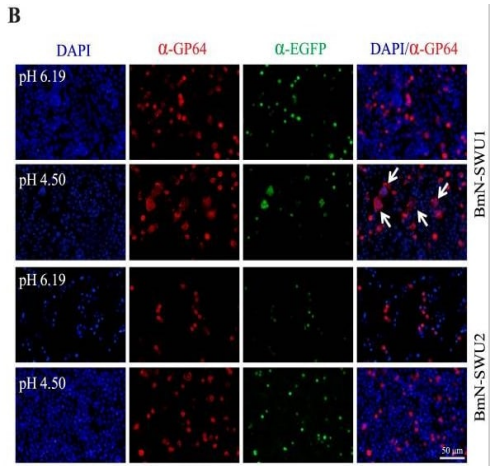
Lane 1 : Untransfected HEK-293E (human epithelial cell line from embryonic kidney) whole cell lysate at 40 µg
Lane 2 : Empty vector control at 40 µg
Lane 3 : H3-GFP at 40 µg
Lane 4 : H3-GFP at 20 µg
Lane 5 : H3-GFP at 10 µg
Lane 6 : p65-GFP at 40 µg
Lane 7 : p65-YFP at 40 µg
Lane 8 : H3-mCherry at 40 µg
Lane 9 : 53 kDa recombinant protein consisting multiple epitope tags at 0.025 µg

Secondary
All lanes : Goat anti-Mouse IgG (H+L) (HRP) at 1/4000 dilution

Developed using the ECL technique.

Predicted band size: 27 kDa

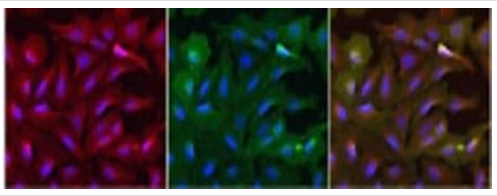
Western Blot was performed using ab184601 by loading whole cell extracts of untransfected and transiently transfected HEK-293E. Lysates were electrophoresed using a 4-12% Bis-Tris Protein Gel. Resolved proteins were then transferred onto a nitrocellulose membrane using a dry blotting system. A ~45 kDa band of H3-GFP and a ~92 kDa band of p65-GFP were observed in transfected lysates. A 53 kDa recombinant protein consisting multiple epitope tags was used as a positive control for GFP detection. This product also detects Yellow Fluorescent Protein (YFP), a variant of GFP as observed in Lane 7. No cross-reactivity was seen with mCherry (RFP family) expressing lysate.



Immunocytochemistry/ Immunofluorescence - Anti-EGFP antibody [F56-6A1.2.3] (ab184601)

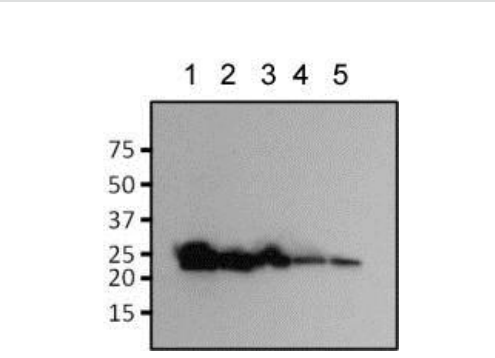
Detection of GP64 expression and fusogenicity.

(B) Syncytium formation assay of BmN-SWU1 or BmN-SWU2 cells transfected with 1 µg pIZ-GP64 plasmid. At 48 h p.t., cells were incubated in TC-100 medium at a normal (6.19) or low (4.8) pH level for 10 min. Syncytium formation was examined 12 h later by detecting GP64 and EGFP (ab184601) using indirect immunofluorescence microscopy. Nuclei were stained with DAPI (blue).



Immunocytochemistry/ Immunofluorescence - Anti-EGFP antibody [F56-6A1.2.3] (ab184601)

Immunofluorescent analysis of U-2 OS (Human bone osteosarcoma epithelial cell line) cells transfected with an EGFR-EGFP fusion protein (formalin-fixed, 0.1% Triton X-100 permeabilized) using either natural fluorescence (green) or an anti-EGFP antibody (red). Cells were then labeled with ab184601 at 1/20 dilution followed with DyLight 550 goat anti-mouse IgG secondary antibody at 1/200 dilution. Nuclei (blue) were stained with Hoechst 33342 dye. 20X magnification.



Western blot - Anti-EGFP antibody [F56-6A1.2.3] (ab184601)

All lanes : Anti-EGFP antibody [F56-6A1.2.3] (ab184601) at 1/1000 dilution

Lane 1 : Recombinant GFP at 1 µg
Lane 2 : Recombinant GFP at 0.5 µg
Lane 3 : Recombinant GFP at 0.25 µg
Lane 4 : Recombinant GFP at 0.125 µg
Lane 5 : Recombinant GFP at 0.0625 µg

Secondary
All lanes : goat anti-mouse IgG-HRP at 1/15000 dilution

Developed using the ECL technique.

Predicted band size: 27 kDa

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

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