# abcam

## Product datasheet

# Anti-GBF1 antibody ab86071

★★★★★ **4 Abreviews**    **12 References**    5 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-GBF1 antibody |
| **Description** | Rabbit polyclonal to GBF1 |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** ICC/IF, WB, IP, IHC-P |
| **Species reactivity** | **Reacts with:** Mouse, Human |
| | **Predicted to work with:** Rat, Guinea pig, Chimpanzee, Rhesus monkey, Chinese hamster, Orangutan ⚠ |
| **Immunogen** | Synthetic peptide within Human GBF1 aa 1800 to the C-terminus (C terminal). The exact immunogen sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support team to discuss your requirements.<br>Database link: **Q92538** |
| **Positive control** | WB: HeLa, HEK-293T and NIH/3T3 whole cell lysate. ICC/IF: HeLa cells. HeLa cells treated with Exo-1. IHC-P: Human prostate carcinoma tissue. IP: HeLa whole cell lysate. |
| **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.<br><br>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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| **Storage buffer** | pH: 6.8<br>Preservative: 0.09% Sodium azide<br>Constituents: 0.1% BSA, Tris buffered saline |
| **Purity** | Immunogen affinity purified |
| **Clonality** | Polyclonal |

| | |
|---|---|
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee**  Our **Abpromise guarantee** covers the use of ab86071 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 |
|---|---|---|
| **ICC/IF** | ★★★★★ (3) | Use a concentration of 1 µg/ml. |
| **WB** | ★★★★★ (1) | 1/2000 - 1/10000. Predicted molecular weight: 206 kDa. |
| **IP** | | Use at 2-5 µg/mg of lysate. |
| **IHC-P** | | 1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |

## Target

**Function**  Guanine-nucleotide exchange factor (GEF) for members of the Arf family of small GTPases involved in trafficking in the early secretory pathway; its GEF activity initiates the coating of nascent vesicles via the localized generation of activated ARFs through replacement of GDP with GTP. Recruitment to cis-Golgi membranes requires membrane association of Arf-GDP and can be regulated by ARF1, ARF3, ARF4 and ARF5. Involved in the recruitment of the COPI coat complex to the endoplasmic reticulum exit sites (ERES), and the endoplasmic reticulum-Golgi intermediate (ERGIC) and cis-Golgi compartments which implicates ARF1 activation. Involved in COPI vesicle-dependent retrograde transport from the ERGIC and cis-Golgi compartments to the endoplasmatic reticulum (ER) (PubMed:16926190, PubMed:17956946, PubMed:18003980, PubMed:12047556, PubMed:12808027, PubMed:19039328, PubMed:24213530). Involved in the trans-Golgi network recruitment of GGA1, GGA2, GGA3, BIG1, BIG2, and the AP-1 adaptor protein complex related to chlathrin-dependent transport; the function requires its GEF activity (probably at least in part on ARF4 and ARF5) (PubMed:23386609). Has GEF activity towards ARF1 (PubMed:15616190). Has in vitro GEF activity towards ARF5 (By similarity). Involved in the processing of PSAP (PubMed:17666033). Required for the assembly of the Golgi apparatus (PubMed:12808027, PubMed:18003980). The AMPK-phosphorylated form is involved in Golgi disassembly during mitotis and under stress conditions (PubMed:18063581, PubMed:23418352). May be involved in the COPI vesicle-dependent recruitment of PNPLA2 to lipid droplets; however, this function is under debate (PubMed:19461073, PubMed:22185782). In neutrophils, involved in G protein-coupled receptor (GPCR)-mediated chemotaxis und superoxide production. Proposed to be recruited by phosphatidylinositol-phosphates generated upon GPCR stimulation to the leading edge where it recruits and activates ARF1, and is involved in recruitment of GIT2 and the NADPH oxidase complex (PubMed:22573891).

**Tissue specificity**  Ubiquitous.

**Sequence similarities**  Contains 1 SEC7 domain.

**Domain**  The DCB (dimerization and cyclophiln-binding) and HUS (homology upstream of Sec7) domains are necessary for dimerization. The DCB domain is proposed to support constitutive homodimerization; the HUS domain interacts with the DCB domain which may occur
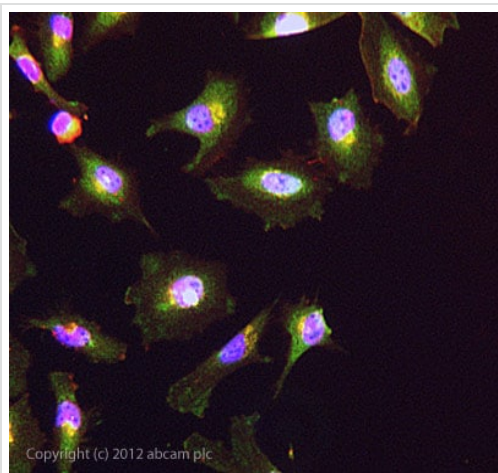
intramolecular or intermolecuar.

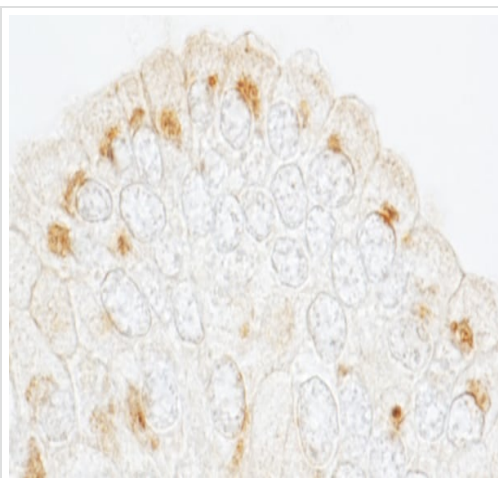| | |
|---|---|
| **Post-translational modifications** | AMPK-mediated phosphorylation at Thr-1337 is induced by 2-deoxyglucose (2-DG) and AICA ribonucleotide, and occurs during mitosis leading to membrane disassociation and inactivation of ARF1 during mitosis. |
| **Cellular localization** | Golgi apparatus, cis-Golgi network. Endoplasmic reticulum-Golgi intermediate compartment. Golgi apparatus, trans-Golgi network. Cytoplasm. Lipid droplet. Membrane. Cycles rapidly on and off early Golgi membranes (PubMed:15616190). Stabilized on membranes when complexed with ARF1-GDP and is released from both ARF1 and membranes after it catalyzes GDP displacement and ARF1 binds GTP. Continuous cycles of recruitment and dissociation of GBF1 to membranes are required for sustained ARF activation and COP I recruitment (PubMed:15813748). In neutrophils is translocated from the Golgi to the leading edge upon GPCR stimulation (PubMed:22573891). Localization to lipid droplets is questionable (PubMed:22185782). |

## Images



ab86071 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab86071 at 1µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit (**ab96899**) IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

Immunocytochemistry/ Immunofluorescence - Anti-GBF1 antibody (ab86071)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling GBF1 with ab86071 at 1/200 (1µg/ml). Detection: DAB.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GBF1 antibody (ab86071)
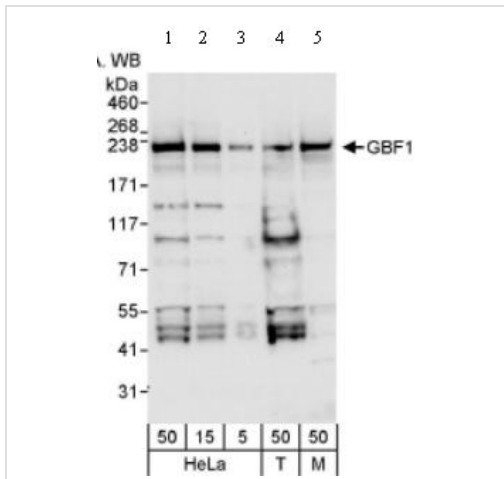
Western blot - Anti-GBF1 antibody (ab86071)

**All lanes :** Anti-GBF1 antibody (ab86071) at 0.04 µg/ml

**Lane 1 :** HeLa whole cell lysate at 50 µg

**Lane 2 :** HeLa whole cell lysate at 15 µg

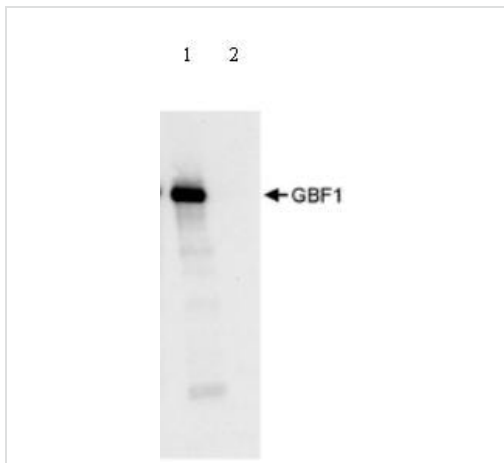**Lane 3 :** HeLa whole cell lysate at 5 µg

**Lane 4 :** 293T cell lysate at 50 µg

**Lane 5 :** mouse NIH3T3 cell lysate at 50 µg

Developed using the ECL technique.

**Predicted band size:** 206 kDa

**Exposure time:** 10 seconds

Immunoprecipitation/ Western Blot of ab86071. Lane 1: ab86071 at 3µg/mg whole cell lysate. Lane 2: Control IgG. ab86071 at 1µg/ml for WB. Whole cell lysate from Hela cells at 1mg for IP, 20% of IP loaded. Chemiluminescence with an exposure time of 3 seconds.



Immunoprecipitation - Anti-GBF1 antibody (ab86071)
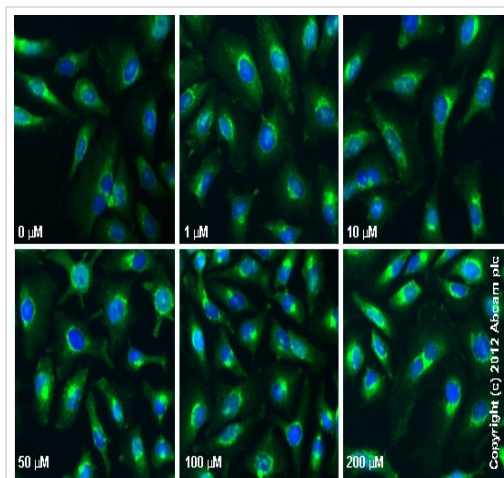


Immunocytochemistry/ Immunofluorescence - Anti-GBF1 antibody (ab86071)

ab86071 staining GBF1 in HeLa cells treated with Exo-1 (**ab120292**), by ICC/IF. Increase in GBF1 expression correlates with increased concentration of Exo-1 as described in literature. The cells were incubated at 37°C for 5 minutes in media containing different concentrations of **ab120292** ( Exo-1) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab86071 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

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