abcam

ab270614

Anti-TFEB antibody [EPR22940-151] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR22940-151
Purification technique	Affinity purification Protein A
Concentration	0.983 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information TFEB

Function

Transcription factor that acts as a master regulator of lysosomal biogenesis, autophagy, lysosomal exocytosis, lipid catabolism, energy metabolism and immune response (PubMed:21617040, PubMed:22343943, PubMed:22576015, PubMed:22692423, PubMed:25720963, PubMed:30120233, PubMed:31672913, PubMed:32612235, PubMed:32753672, PubMed:35662396, PubMed:36697823, PubMed:36749723, PubMed:37079666). Specifically recognizes and binds E-box sequences (5'-CANNTG-3'); efficient DNA-binding requires dimerization with itself or with another MiT/TFE family member such as TFE3 or MITF (PubMed:1748288, PubMed:19556463, PubMed:29146937). Involved in the cellular response to amino acid availability by acting downstream of MTOR: in the presence of nutrients, TFEB phosphorylation by MTOR promotes its cytosolic retention and subsequent inactivation (PubMed:21617040, PubMed:22343943, PubMed:22576015, PubMed:22692423, PubMed:25720963, PubMed:32612235, PubMed:32753672, PubMed:35662396, PubMed:36697823). Upon starvation or lysosomal stress, inhibition of MTOR induces TFEB dephosphorylation, resulting in nuclear localization and transcription factor activity (PubMed:22343943, PubMed:22576015, PubMed:22692423, PubMed:25720963, PubMed:32612235, PubMed:32753672, PubMed:35662396, PubMed:36697823). Specifically recognizes and binds the CLEAR-box sequence (5'-GTCACGTGAC-3') present in the regulatory region of many lysosomal genes, leading to activate their expression, thereby playing a central role in expression of lysosomal genes (PubMed:19556463, PubMed:22692423). Regulates lysosomal positioning in response to nutrient deprivation by promoting the expression of PIP4P1 (PubMed:29146937). Acts as a positive regulator of autophagy by promoting expression of genes involved in autophagy (PubMed:21617040, PubMed:22576015, PubMed:23434374, PubMed:27278822). In association with TFE3, activates the expression of CD40L in T-cells, thereby playing a role in T-cell-dependent antibody responses in activated CD4(+) T-cells and thymus-dependent humoral immunity (By similarity). Specifically recognizes the gamma-E3 box, a subset of E-boxes, present in the heavy-chain immunoglobulin enhancer (PubMed:2115126). Plays a role in the signal transduction processes required for normal vascularization of the placenta (By similarity). Involved in the immune response to infection by the bacteria S.aureus, S.typhimurium or S.enterica: infection promotes itaconate production, leading to alkylation, resulting in nuclear localization and transcription factor activity (PubMed:35662396). Itaconate-mediated alkylation activates TFEB-dependent lysosomal biogenesis, facilitating the bacteria clearance during the antibacterial innate immune response (PubMed:35662396). In association with ACSS2, promotes the expression of genes involved in lysosome biogenesis and both autophagy upon glucose deprivation (PubMed:28552616).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab270614 is the carrier-free version of ab270604.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

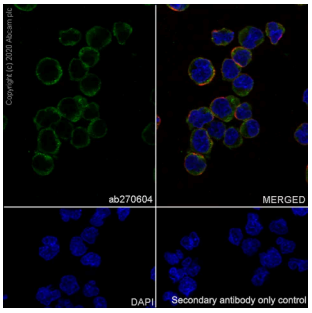
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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12 product images

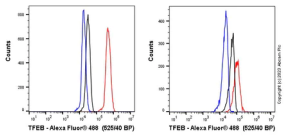


Immunocytochemistry/ Immunofluorescence - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-TFEB antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji cells labeling TFEB with ab270604 at 1/50 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in Raji cells. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).



Flow Cytometry (Intracellular) - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Flow Cytometry (Intracellular) staining using rabbit Anti-TFEB antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).

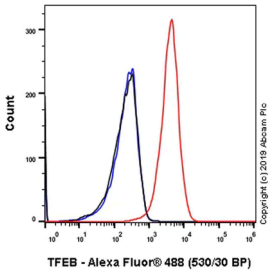
Flow cytometry overlay histogram showing left Raji positive cells and right negative MCF7 stained with ab270604 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein

interaction followed by the antibody (ab270604) (1x 106 in 100µl at 0.2µg/ml (1/10350)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

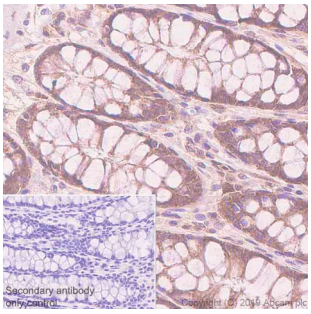
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Flow Cytometry (Intracellular) - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Flow Cytometry (Intracellular) staining using rabbit Anti-TFEB antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling TFEB with ab270604 at 1/500 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).



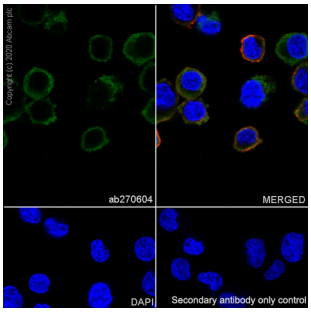
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TFEB antibody

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling TFEB with ab270604 at 1/500 followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in human colon tissue is observed (PMID:30519051). The section was incubated with ab270604 for 20 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).

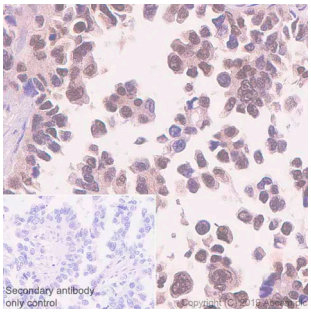


Immunocytochemistry/ Immunofluorescence - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-TFEB antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized IM-9 cells labeling TFEB with ab270604 at 1/50 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in IM-9 cells. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).



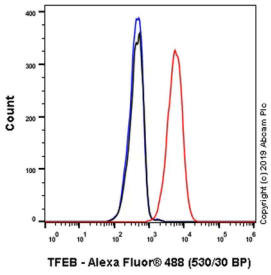
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TFEB antibody

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling TFEB with ab270604 at 1/500 followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in human lung cancer tissue is observed (PMID:26264650). The section was incubated with ab270604 for 20 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

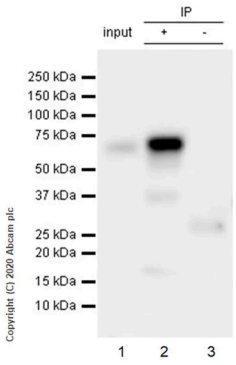
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).



Flow Cytometry (Intracellular) - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Flow Cytometry (Intracellular) staining using rabbit Anti-TFEB antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized IM-9 (Human multiple myeloma B Lymphoblast) cells labeling TFEB with ab270604 at 1/500 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor®488, ab150077) at 1/2000 dilution was used as the secondary antibody. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).



Immunoprecipitation - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB was immunoprecipitated from 0.35 mg Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate with ab270604 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab270604 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/1000 dilution.
Lane 1: Raji whole cell lysate.

Lane 2: ab270604 IP in Raji whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab270604 in Raji whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

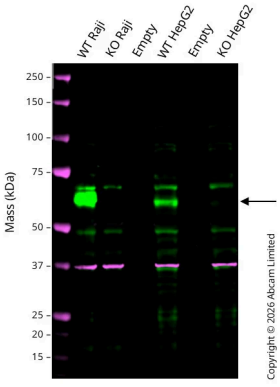
Exposure time: 3 secs.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).

All lanes:
Immunoprecipitation - Anti-TFEB antibody [EPR22940-151] (ab270604)

Predicted band size: 53 kDa

Observed band size: 66 kDa



Western blot - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

TFEB Western blot staining using rabbit Anti-TFEB antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab270604).

Western blot: Anti-TFEB antibody [EPR22940-151] ab270604 staining at 1/500 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 60 kDa in Wild-type Raji cell lysates with no signal observed at this size in TFEB knockout Raji cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 100% LICOR in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-TFEB antibody [EPR22940-151] (ab270604) at 1/500 dilution

Lane 1:
Wild-type Raji cell lysates at 20 µg

Lane 2:
Western blot - Human TFEB knockout Raji cell line (ab275832) at 20 µg

Lanes 3 and 5:
EMPTY

Lane 4:
Human wild-type Hep G2 cell line at 40 µg

Lane 6:

Western blot - Human TFEB knockout Hep G2 cell line (ab277871) at 40 µg

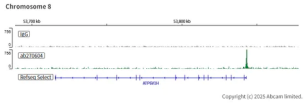
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 60 kDa

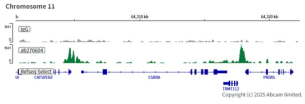
Observed band size: 60 kDa



ChIC/CUT&RUN sequencing - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (,ab270604).

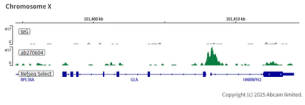
ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 Raji (human Burkitt's lymphoma B lymphocyte) cells and 5 µg of ab270604 [EPR22940-151]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIC/CUT&RUN sequencing - Anti-TFEB antibody [EPR22940-151] - BSA and Azide free (ab270614)

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Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.