abcam

Product datasheet

Anti-TFEB antibody [EPR22941-6] ab267351

KO VALIDATED Recombinant RabMAb

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Overview

| Product name | Anti-TFEB antibody [EPR22941-6] |
|---------------------|---|
| Description | Rabbit monoclonal [EPR22941-6] to TFEB |
| Host species | Rabbit |
| Tested applications | Suitable for: WB, IP, ICC/IF, IHC-P, Flow Cyt (Intra) |
| Species reactivity | Reacts with: Human |
| Immunogen | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| Positive control | WB: Raji and IM-9 lysates. IHC-P: Human colon, Human lung cancer and Human breast cancer tissues. ICC/IF: Raji and IM-9 cells. Flow Cyt (intra): IM-9 cells. IP: Raji and IM-9 cells. |
| General notes | 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 production For more information <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. |

| 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 | pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA |
| Purity | Protein A purified |
| Clonality | Monoclonal |
| Clone number | EPR22941-6 |
| lsotype | lgG |

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab267351 in the following tested applications.

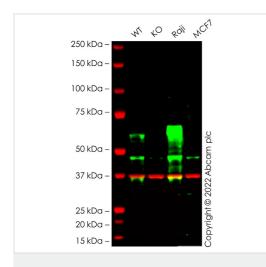
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|------------------|----------------------|---|
| WB | ★ ★ ★ ★ ★ (1) | 1/1000. Detects a band of approximately 66 kDa (predicted molecular weight: 53 kDa). |
| IP | | 1/30. |
| ICC/IF | | 1/50. |
| IHC-P | | 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| Flow Cyt (Intra) | | 1/500. |

Target

| Sequence similaritiesBelongs to the MiT/TFE family. Contains 1 basic helix-loop-helix (bHLH) domain.DomainThe leucin zipper region is essential for homo- or heterodimerization and high-affinity DNA binding. DNA binding is mediated by the basic region.Post-translational modificationsSumoylated; does not affect dimerization with MITF. | Function | Transcription factor that specifically recognizes and binds E-box sequences (3'-CANNTG-5'). Efficient DNA-binding requires dimerization with itself or with another MiT/TFE family member such as TFE3 or MITF. 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. 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. It thereby plays a central role in expression of lysosomal genes. Specifically recognizes the gamma-E3 box, a subset of E-boxes, present in the heavy- chain immunoglobulin enhancer. Plays a role in the signal transduction processes required for normal vascularization of the placenta. |
|---|-----------------------|--|
| Post-translational Sumoylated; does not affect dimerization with MITF. | Sequence similarities | |
| ,, | Domain | |
| | | Sumoylated; does not affect dimerization with MITF. |
| Cellular localizationCytoplasm. Nucleus. Mainly present in the cytoplasm. Under aberrant lysosomal storage conditions, it translocates from the cytoplasm to the nucleus. | Cellular localization | |

Images



Western blot - Anti-TFEB antibody [EPR22941-6] (ab267351)

All lanes : Anti-TFEB antibody [EPR22941-6] (ab267351) at 1/1000 dilution

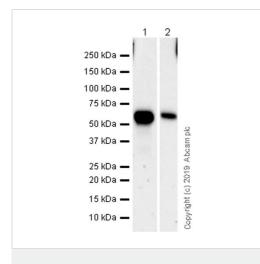
Lane 1 : Wild-type HepG2 cell lysate Lane 2 : TFEB knockout HepG2 cell lysate Lane 3 : Raji cell lysate Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 53 kDa Observed band size: 60 kDa

False colour image of Western blot: Anti-TFEB antibody [EPR22941-6] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab267351 was shown to bind specifically to TFEB. A band was observed at 60 kDa in wild-type HepG2 cell lysates with no signal observed at this size in TFEB knockout cell line ab277871 (knockout cell lysate ab284220). To generate this image, wild-type and TFEB knockout HepG2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk 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 IgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-TFEB antibody [EPR22941-6] (ab267351) All lanes : Anti-TFEB antibody [EPR22941-6] (ab267351) at 1/1000 dilution

Lane 1 : Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lane 2 : IM-9 (human multiple myeloma B Lymphoblast whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

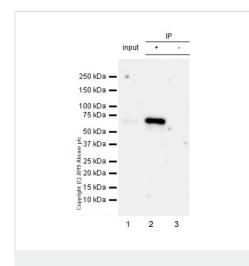
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 53 kDa Observed band size: 66 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

The molecular weight observed is consistent with what has been described in the literature (PMID:30519051).



Immunoprecipitation - Anti-TFEB antibody [EPR22941-6] (ab267351) TFEB was immunoprecipitated from 0.35 mg IM-9 (human multiple myeloma B Lymphoblast) whole cell lysate with ab267351 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267351 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: IM-9 (human multiple myeloma B Lymphoblast) whole cell lysate 10ug

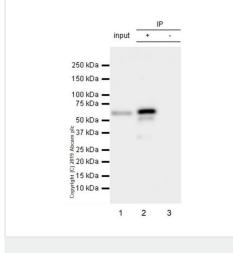
Lane 2: ab267351 IP in IM-9 whole cell lysate

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab267351 in IM-9 whole cell lysate

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

Exposure time: 2 min.

The weak band around 50kDa is one isoform of TFEB(PMID:28993453).



Immunoprecipitation - Anti-TFEB antibody [EPR22941-6] (ab267351) TFEB was immunoprecipitated from 0.35 mg Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate with ab267351 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267351 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate 10ug

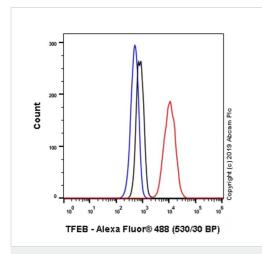
Lane 2: ab267351 IP in Raji whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab267351 in Raji whole cell lysate

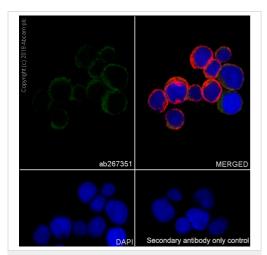
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds.

The weak band around 50kDa is one isoform of TFEB(PMID:28993453).

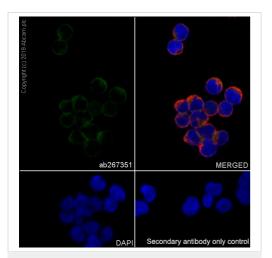


Flow Cytometry (Intracellular) - Anti-TFEB antibody [EPR22941-6] (ab267351) Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized IM-9 (human multiple myeloma B Lymphoblast) cells labelling TFEB with ab267351 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled 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.



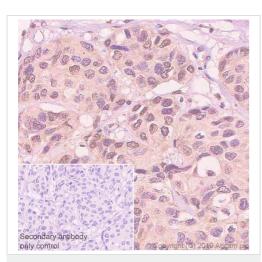
Immunocytochemistry/ Immunofluorescence - Anti-TFEB antibody [EPR22941-6] (ab267351) Immunofluorescent analysis of 100% Methanol-fixed IM-9 (human multiple myeloma B Lymphoblast) cells labelling TFEB with ab267351 at 1/50 dilution, followed by <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in IM-9 cells. 100% methanol fixation is recommended. <u>ab195889</u> 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 <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-TFEB antibody [EPR22941-6] (ab267351) Immunofluorescent analysis of 100% Methanol-fixed Raji (human Burkitt's lymphoma B lymphocyte) cells labelling TFEB with ab267351 at 1/50 dilution, followed by <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in Raji cells. 100% methanol fixation is recommended. <u>ab195889</u> 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 <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.

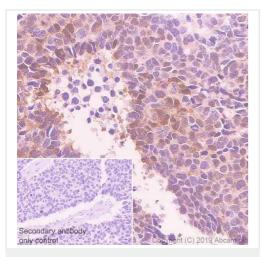


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody [EPR22941-6] (ab267351)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling TFEB with ab267351 at 1/500 dilution (1.042ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining in human breast cancer (PMID:28017540). The section was incubated with **ab256492** for 15 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 Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

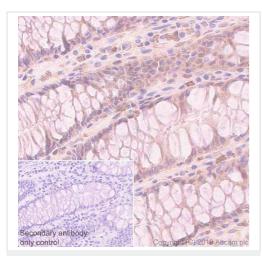


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody [EPR22941-6] (ab267351)

Immunohistochemical analysis of paraffin-embedded Human lung cancer tissue labeling TFEB with ab267351 at 1/500 dilution (1.042ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining in human lung cancer (PMID: 26264650) The section was incubated with <u>ab256492</u> for 15 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 Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody [EPR22941-6] (ab267351)

Why choose a recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant technology reproducible results Success from the Ethical standards first experiment compliant Confirmed Animal-free specificity production Anti-TFEB antibody [EPR22941-6] (ab267351) Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling TFEB with ab267351 at 1/500 dilution (1.042ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining human colon (PMID:30519051). The section was incubated with **ab256492** for 15 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 Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

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