abcam

Product datasheet

Anti-TFEB antibody [EPR22941-6] ab267351



ייבער RabMAb

★★★★★ 1 Abreviews 2 References 画像数 11

製品の概要

製品名 Anti-TFEB antibody [EPR22941-6]

製品の詳細 Rabbit monoclonal [EPR22941-6] to TFEB

由来種 Rabbit

アプリケーション 適用あり: WB, IP, ICC/IF, IHC-P, Flow Cyt (Intra)

種交差性 交差種: Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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 see here.

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

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

クローン名 EPR22941-6

アイソタイプ lgG

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab267351の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

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.

配列類似性

Belongs to the MiT/TFE family.

Contains 1 basic helix-loop-helix (bHLH) domain.

ドメイン

The leucin zipper region is essential for homo- or heterodimerization and high-affinity DNA

binding. DNA binding is mediated by the basic region.

翻訳後修飾

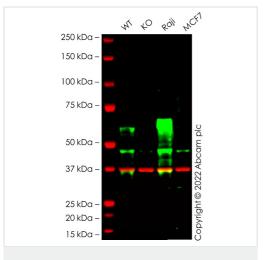
Sumoylated; does not affect dimerization with MITF.

細胞内局在

Cytoplasm. Nucleus. Mainly present in the cytoplasm. Under aberrant lysosomal storage

conditions, it translocates from the cytoplasm to the nucleus.

画像



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

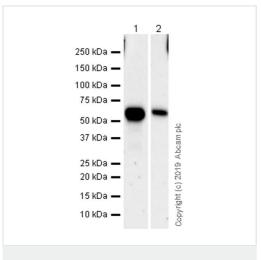
Lane 4: MCF7 cell lysate

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

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

Lysates/proteins at 10 µg per lane.

Secondary

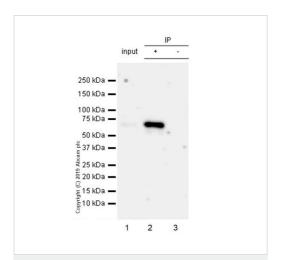
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

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

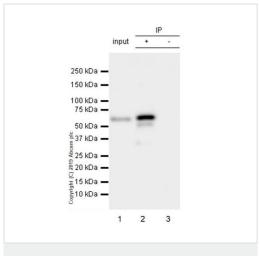
Lane 2: ab267351 IP in IM-9 whole cell lysate

Lane 3: Rabbit monoclonal IgG (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

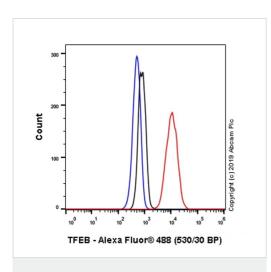
Lane 2: ab267351 IP in Raji whole cell lysate

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

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

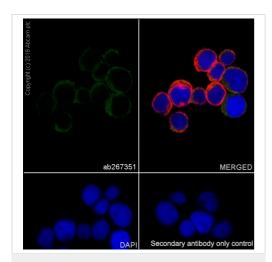
Exposure time: 15 seconds.

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



Flow Cytometry (Intracellular) - Anti-TFEB antibody [EPR22941-6] (ab267351)

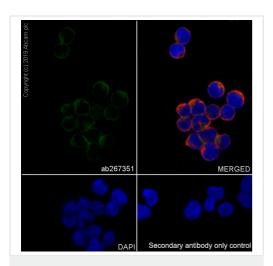
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized IM-9 (human multiple myeloma B Lymphoblast) cells labelling TFEB with ab267351 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells 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 ab150077 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. 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).

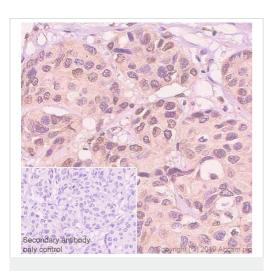
Secondary antibody only control: Secondary antibody is <u>ab150077</u>
AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.



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 **ab150077** 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. **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 <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution.

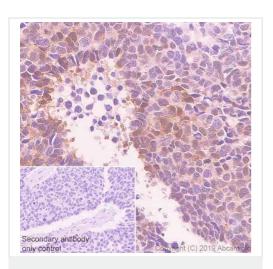


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody
[EPR22941-6] (ab267351)

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 (ab209101).

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

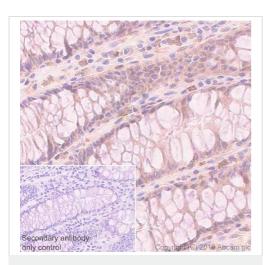


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody
[EPR22941-6] (ab267351)

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 (ab209101). Positive staining in human lung cancer (PMID: 26264650) 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 (ab209101).

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

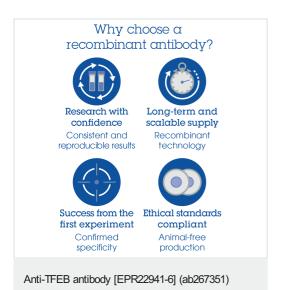


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TFEB antibody
[EPR22941-6] (ab267351)

Immunohistochemical analysis of paraffin-embedded Human 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 (ab209101).

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



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