abcam

Product datasheet

Anti-ULK3 antibody [EPR4888] ab124947

КО 評価済 มาวนราวง RabMAb

<u>2 References</u> 画像数 8

製品の概要

| 製品名 | Anti-ULK3 antibody [EPR4888] |
|--------------|---|
| 製品の詳細 | Rabbit monoclonal [EPR4888] to ULK3 |
| 由来種 | Rabbit |
| アプリケーション | 適用あり: Flow Cyt (Intra), WB, ICC/IF, IHC-P 適用なし: IP |
| 種交差性 | 交差種: Human 非交差種: Mouse, Rat |
| 免疫原 | Synthetic peptide within Human ULK3 aa 400-500 (C terminal). The exact sequence is proprietary. Database link: <u>Q6PHR2-3</u> |
| ポジティブ・コントロール | IHC-P: Human bladder carcinoma and human colon tissue; WB: Wild-type HAP1 whole cell lysate; HEK-293 and LNCaP whole cell lysates. Human heart and testis tissue lysates. ICC/IF: LNCaP cells; Flow Cyt (intra): LNCaP 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 <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>. |

| 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: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS |

| 精製度 | Protein A purified |
|--------|--------------------|
| ポリ/モノ | モノクローナル |
| クローン名 | EPR4888 |
| アイソタイプ | lgG |

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab124947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。

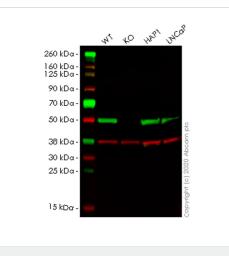
| アプリケーション | Abreviews | 特記事項 |
|------------------|-----------|--|
| Flow Cyt (Intra) | | 1/10 - 1/100. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| WB | | 1/1000 - 1/10000. Predicted molecular weight: 53 kDa. |
| ICC/IF | | 1/250. |
| IHC-P | | 1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |

追加情報

Is unsuitable for IP.

| 報 |
|---|
| |

| 機能 | Serine/threonine protein kinase which enhances GLI1 and GLI2 transcriptional activity and consequently positively regulates GLI-dependent SHH signaling. May exert this function by promoting GLI1 nuclear localization. Phosphorylates in vitro GLI2, as well as GLI1 and GLI3, although less efficiently. |
|-------|--|
| 組織特異性 | Widely expressed. Highest levels observed in fetal brain. In adult tissues, high levels in brain, liver and kidney, moderate levels in testis and adrenal gland and low levels in heart, lung, stomach, thymus, prostate and placenta. In the brain, highest expression in the hippocampus, high levels also detected in the cerebellum, olfactory bulb and optice nerve. In the central nervous system, lowest levels in the spinal cord. |
| 配列類似性 | Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. APG1/unc-51/ULK1 subfamily. Contains 2 MIT domains. Contains 1 protein kinase domain. |
| 翻訳後修飾 | Autophosphorylated in vitro. |
| 細胞内局在 | Cytoplasm. |
| 製品の状態 | There are 3 isoforms produced by alternative splicing. |



Western blot - Anti-ULK3 antibody [EPR4888] (ab124947) All lanes : Anti-ULK3 antibody [EPR4888] (ab124947) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate Lane 2 : ULK3 knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate Lane 3 : HAP1 whole cell lyate

Lane 4 : LNCaP (Human prostate cancer cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

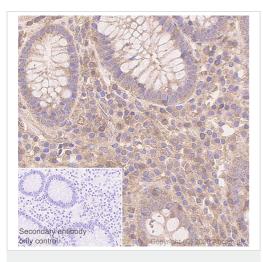
Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 53 kDa Observed band size: 55 kDa

Lanes 1-4: Merged signal (red and green). Green - ab124947 observed at 55 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

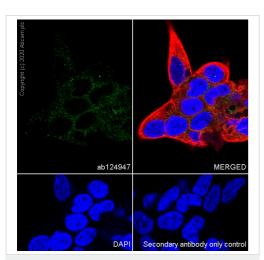
ab124947 Anti-ULK3 antibody [EPR4888] was shown to specifically react with ULK3 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <u>ab266152</u> (knockout cell lysate <u>ab258271</u>) was used. Wild-type and ULK3 knockout samples were subjected to SDS-PAGE. ab124947 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



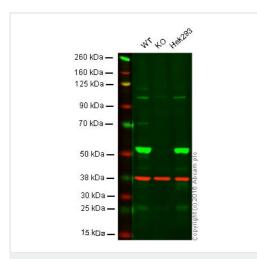
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue sections labeling ULK3 with purified ab124947 at 1/50 dilution (7.84 µg/mL). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ULK3 antibody [EPR4888] (ab124947)



Immunocytochemistry/ Immunofluorescence - Anti-ULK3 antibody [EPR4888] (ab124947) Immunocytochemistry/Immunofluorescence analysis of LNCaP (Human prostate carcinoma epithelial cell) cells labeling ULK3 with Purified ab124947 at 1:50 dilution (7.8 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-ULK3 antibody [EPR4888] (ab124947)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg) Lane 2: ULK3 knockout HAP1 whole cell lysate (20 µg) Lane 3: Hek293 whole cell lysate (20 µg) Lanes 1 - 3: Merged signal (red and green). Green - ab124947 observed at 55 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab124947 (unpurified) was shown to specifically react with ULK3 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when ULK3 knockout samples were examined. Wild-type and ULK3 knockout samples were subjected to SDS-PAGE. Ab124947 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) **ab216773** and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) **ab216776** secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-ULK3 antibody [EPR4888] (ab124947) at 1/1000 dilution (Purified)

Lane 1 : Human heart lysate

Lane 2 : Human testis lysate

Lane 3 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate

Lane 4 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

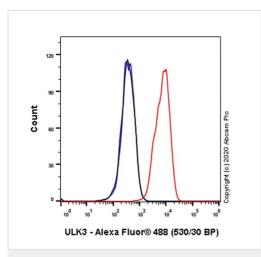
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

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

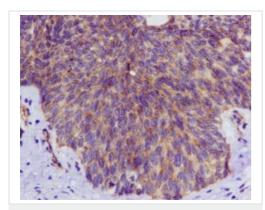


(ab124947)





Flow Cytometry (Intracellular) - Anti-ULK3 antibody [EPR4888] (ab124947) Intracellular Flow Cytometry analysis of LNCaP (Human prostate carcinoma epithelial cell) cells, labeling ULK3 with Purified ab124947 at 1/40 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ULK3 antibody [EPR4888] (ab124947) ab124947 (unpurified), at a 1/50 dilution, staining ULK3 in paraffin embedded Human bladder carcinoma tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

Why choose α recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant reproducible results technology Success from the Ethical standards first experiment compliant Animal-free Confirmed specificity production

Anti-ULK3 antibody [EPR4888] (ab124947)

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