# abcam

## Product datasheet

## Human RAB7A (RAB7) knockout HeLa cell lysate ab263831

### 画像数3

#### 製品の概要

製品名 Human RAB7A (RAB7) knockout HeLa cell lysate

製品の概要

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HeLa
Organism Human

**Mutation description** Knockout achieved by using CRISPR/Cas9, 25 bp deletion in exon2.

Passage number <20

**Knockout validation** Sanger Sequencing, Western Blot (WB)

 $\label{eq:Reconstitution notes} \textbf{To use as WB control, resuspend the lyophilizate in 50 $\mu$L of LDS* Sample Buffer to have a final $\mu$L of LDS* Sample$ 

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

 $\hbox{$^*$Usage of SDS sample buffer is not recommended with these lyophilized lysates.}$ 

特記事項

**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared.

**User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. See here for more information on knockout cell lysates.

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**アプリケーション** 適用あり: WB

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#### 製品の特性

#### 保存方法

Store at -80°C. Please refer to protocols.

	1 kit
ab255527 - Human RAB7A knockout HeLa cell lysate	1 x 100μg
ab255929 - Human wild-type HeLa cell lysate	1 x 100μg

**Cell type** epithelial

**Disease** Adenocarcinoma

**Gender** Female

**STR Analysis** Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18

TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

#### ターゲット情報

#### 機能

Key regulator in endo-lysosomal trafficking. Governs early-to-late endosomal maturation, microtubule minus-end as well as plus-end directed endosomal migration and positioning, and endosome-lysosome transport through different protein-protein interaction cascades. Plays a central role, not only in endosomal traffic, but also in many other cellular and physiological events, such as growth-factor-mediated cell signaling, nutrient-transportor mediated nutrient uptake, neurotrophin transport in the axons of neurons and lipid metabolism. Also involved in regulation of some specialized endosomal membrane trafficking, such as maturation of melanosomes, pathogen-induced phagosomes (or vacuoles) and autophagosomes. Plays a role in the maturation and acidification of phagosomes that engulf pathogens, such as S.aureus and M.tuberculosis. Plays a role in the fusion of phagosomes with lysosomes. Plays important roles in microbial pathogen infection and survival, as well as in participating in the life cycle of viruses. Microbial pathogens possess survival strategies governed by RAB7A, sometimes by employing RAB7A function (e.g. Salmonella) and sometimes by excluding RAB7A function (e.g. Mycobacterium). In concert with RAC1, plays a role in regulating the formation of RBs (ruffled borders) in osteoclasts. Controls the endosomal trafficking and neurite outgrowth signaling of NTRK1/TRKA. Regulates the endocytic trafficking of the EGF-EGFR complex by regulating its lysosomal degradation.

組織特異性

関連疾患

 $\label{thm:pressed} \mbox{Widely expressed; high expression found in skeletal muscle.}$ 

Defects in RAB7A are the cause of Charcot-Marie-Tooth disease type 2B (CMT2B) [MIM:600882]; also known as hereditary motor and sensory neuropathy II (HMSN2). CMT2B is a form of Charcot-Marie-Tooth disease, the most common inherited disorder of the peripheral nervous system. Charcot-Marie-Tooth disease is classified in two main groups on the basis of electrophysiologic properties and histopathology: primary peripheral demyelinating neuropathy or CMT1, and primary peripheral axonal neuropathy or CMT2. Neuropathies of the CMT2 group are characterized by signs of axonal regeneration in the absence of obvious myelin alterations, normal or slightly reduced nerve conduction velocities, and progressive distal muscle weakness and atrophy. CMT2B is clinically characterized by marked distal muscle weakness and a high frequency of foot ulcers, infections and amputations of the toes. CMT2B inheritance is autosomal dominant.

配列類似性

Belongs to the small GTPase superfamily. Rab family.

細胞内局在

Late endosome. Lysosome. Cytoplasmic vesicle > phagosome. Melanosome. Cytoplasmic

vesicle > phagosome membrane. Co-localizes with OSBPL1A at the late endosome. Found in the ruffled border (a late endosomal-like compartment in the plasma membrane) of bone-resorbing osteoclasts. Recruited to phagosomes containing S.aureus or Mycobacterium.

#### アプリケーション

**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ab263831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		Use at an assay dependent concentration.

#### 画像



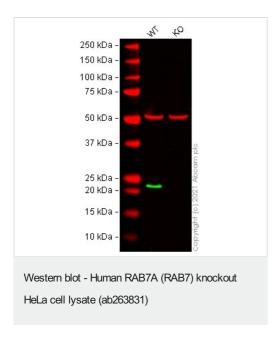
Western blot - Human RAB7A (RAB7) knockout HeLa cell lysate (ab263831)

Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: RAB7A knockout HeLa cell lysate 20 µg

**Lanes 1 - 2:** Merged signal (red and green). Green - <u>ab137029</u> observed at 23 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab137029 was shown to react with RAB7 in wild-type HeLa cells in Western blot with loss of signal observed in RAB7A knockout cell line ab255423 (RAB7A knockout cell lysate ab263831). Wild-type HeLa and RAB7A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab137029 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

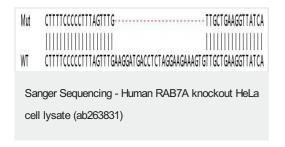


**Lane 1:** Wild-type HeLa cell lysate 20 μg **Lane 2:** RAB7A knockout HeLa cell lysate 20 μg

Lanes 1 - 2: Merged signal (red and green). Green - <u>ab126712</u> observed at 23 kDa. Red - loading control <u>ab7291</u> (Mouse anti-

Alpha Tubulin [DM1A]) observed at 55kDa.

ab126712 was shown to react with RAB7 in wild-type HeLa cells in Western blot with loss of signal observed in RAB7A knockout cell line ab255423 (RAB7A knockout cell lysate ab263831). Wild-type HeLa and RAB7A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab126712 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Homozygous: 25 bp deletion in exon2

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