abcam

Product datasheet

Human P4HB knockout A-431 cell lysate ab261696

画像数 2

製品の概要

製品名	Human P4HB knockout A-431 cell lysate
製品の概要	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 20 bp deletion; Frameshift = 99.4%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **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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製品の特性

保存方法	Store at -80°C. Please refer to protocols.

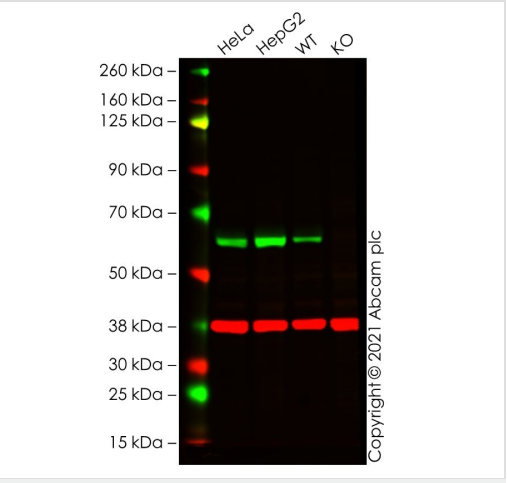
内容	1 kit
ab280446 - Human P4HB knockout A431 cell lysate	1 x 100µg
ab263973 - Human wild-type A-431 cell lysate	1 x 100µg

Cell type	epithelial
Disease	Epidermoid Carcinoma
Gender	Female

ターゲット情報

機能	This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with other chaperones in the structural modification of the TG precursor in hormone biogenesis. Also acts a structural subunit of various enzymes such as prolyl 4-hydroxylase and microsomal triacylglycerol transfer protein MTTP.
配列類似性	Belongs to the protein disulfide isomerase family. Contains 2 thioredoxin domains.
細胞内局在	Endoplasmic reticulum lumen. Melanosome. Cell membrane. Highly abundant. In some cell types, seems to be also secreted or associated with the plasma membrane, where it undergoes constant shedding and replacement from intracellular sources (Probable). Localizes near CD4-enriched regions on lymphoid cell surfaces. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

画像



KO Western blot

Lane 1: HeLa cell lysate, 20 ug

Lane 2: HepG2 cell lysate, 20 ug

Lane 3: Wild-type A431 cell lysate, 20 ug

Lane 4: P4HB knockout A431 cell lysate, 20 ug

False colour image of Western blot: Anti-P4HB antibody [EPR9499] 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, **ab137110** was shown to bind specifically to P4HB. A band was observed at 60 kDa in wild-type HeLa cell lysates with no signal observed at this size in P4HB knockout cell line **ab261887** (knockout cell lysate ab261696). To generate this image, wild-type and P4HB knockout

HeLa 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.

Next Generation Sequencing - Human P4HB knockout A-431 cell lysate (ab261696)

X = 20 bp deletion

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