abcam

Product datasheet

Human CANX (Calnexin) knockout HEK-293T cell lysate ab263805

4 图**像**

概述

产品名称	人CANX (Calnexin) knockout HEK-293T cell裂解物	
产 品概述		
	Knockout cell lysate achieved by CRISPR/Cas9.	
Parental Cell Line	HEK293T	
Organism	Human	
Mutation description	Knockout achieved by using CRISPR/Cas9, 19 bp deletion in exon2.	
Passage number	<20	
Knockout validation	Sanger Sequencing, Western Blot (WB)	
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.	
说 明	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). <i>This means that the protein of interest is denatured.</i> 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.	
	Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.	
	This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses an relevant patents please refer to our limited use license and patent pages .	
经测试应用	适用于: WB	

性能

存放说明

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

组件	1 kit
ab255473 - Human CANX knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type	epithelial	
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12	

靶标		
功能	Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins.	
序列相似性	Belongs to the calreticulin family.	
细 胞定位	Endoplasmic reticulum membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.	

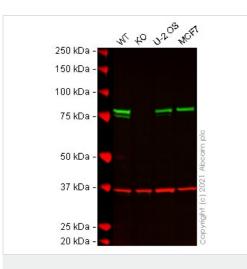
应用

The Abpromise guarantee Abpromise™承诺保证使用ab263805于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration.

图片

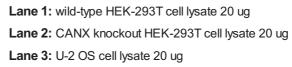


Western blot - Human CANX knockout HEK293T cell lysate (ab263805)

Lane 1: Wild-type HEK-293T cell lysate (20 ug) Lane 2: CANX knockout HEK-293T cell lysate (20 ug) Lane 3: U-2 OS cell lysate (20 ug) Lane 4: MCF7 cell lysate (20 ug)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab92573</u> observed at 80 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

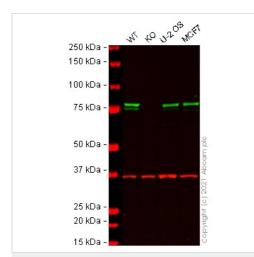
ab92573 was shown to react with Calnexin in wild-type HEK-293T cells in Western blot with loss of signal observed in CANX knockout cell line **ab255368** (CANX knockout cell lysate ab263805). Wild-type HEK-293T and CANX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with **ab92573** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 20000 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 4: MCF7 cell lysate 20 ug

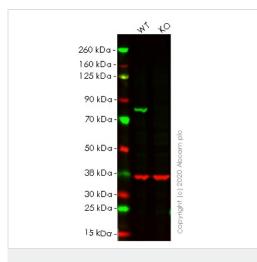
Lanes 1 - 4: Merged signal (red and green). Green - <u>ab238078</u> observed at 80 kDa. Red - loading control <u>ab181602</u> (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

<u>ab238078</u> was shown to react with Calnexin in wild-type HEK-293T cells in Western blot with loss of signal observed in CANX knockout cell line <u>ab255368</u> (CANX knockout cell lysate ab263805). Wild-type HEK-293T and CANX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with <u>ab238078</u> and <u>ab181602</u> (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4 °C at 1 ug/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat



Western blot - Human CANX (Calnexin) knockout HEK293T cell lysate (ab263805)

anti-Mouse IgG H&L (IRDye[®] 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye[®] 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human CANX (Calnexin) knockout HEK293T cell lysate (ab263805) Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: CANX knockout HEK-293T cell lysate (20µg)

Lanes 1-2: Merged signal (red and green). Green - <u>ab133615</u> observed at 90 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab133615 Recombinant Anti-Calnexin antibody [EPR3632] was shown to specifically react with CANX in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255368** (knockout cell lysate ab263805) was used. Wild-type and CANX knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab133615** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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[®] 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Homozygous: 19 bp deletion in exon2

 WT
 AGTAACATACACAGCAACCACTTCCCTTCCATGATCTACACATAAAGAGCAAAAATCAGTT

 Sanger Sequencing - Human CANX knockout

AGTAACATACACAGCAACCACT-----ATAAAGAGCAAAATCAGTT

HEK293T cell lysate (ab263805)

Mut

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