abcam

Product datasheet

Human HLA-A knockout A-431 cell lysate ab261703

3 Images

Overview

Product name	Human HLA-A knockout A-431 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 17 bp deletion; Frameshift = 99.8%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), 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.*
Notes	**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.** 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 and relevant patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Properties

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

Components	1 kit
ab280453 - Human HLA-A 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

Target

Relevance HLA-A belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. Class I molecules play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. They are expressed in nearly all cells. The heavy chain is approximately 45 kDa and its gene contains 8 exons. Exon 1 encodes the leader peptide, exons 2 and 3 encode the alpha1 and alpha2 domains, which both bind the peptide, exon 4 encodes the alpha3 domain, exon 5 encodes the transmembrane region, and exons 6 and 7 encode the cytoplasmic tail. Polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class one molecule. Typing for these polymorphisms is routinely done for bone marrow and kidney transplantation. Hundreds of HLA-A alleles have been described.

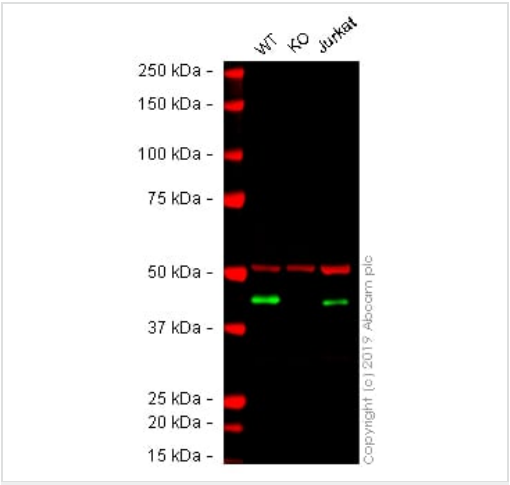
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261703 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 41 kDa.

Images



Western blot - Human HLA-A knockout A-431 cell lysate (ab261703)

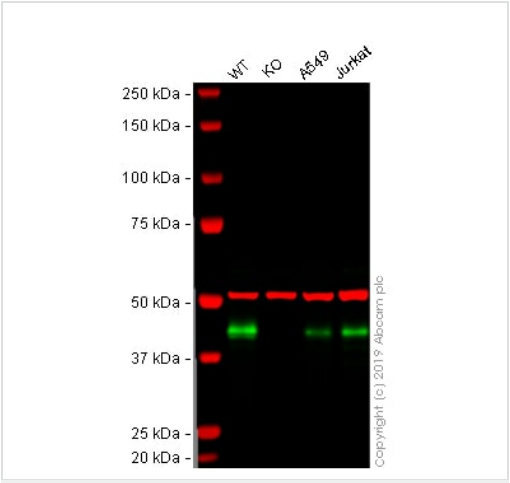
Lane 1: Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2: EPCAM knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lanes 1 - 3: Merged signal (red and green). Green - **ab86597** observed at 40 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab86597 was shown to react with HLA-A in wild-type A-431 cells in Western blot Loss of signal was observed when HLA-A knockout cell line **ab261894** (knockout cell lysate ab261703) was used. Wild-type A-431 and HLA-A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab86597** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 5 µg/ml and a 1 in 20000 dilution respectively. Blots were developed with Goat 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 hour at room temperature before imaging.



Western blot - Human HLA-A knockout A-431 cell lysate (ab261703)

Lane 1: Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

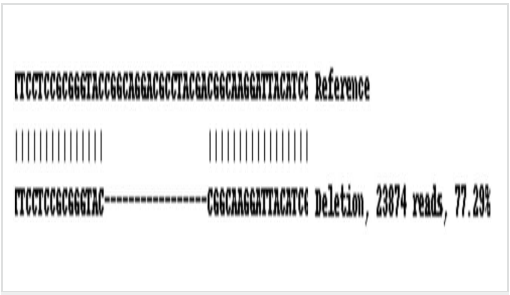
Lane 2: HLA A knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate 20 ug

Lane 3: A549 (Human lung carcinoma cell line) whole cell lysate 20 ug

Lane 4: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab52922** observed at 40 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab52922 was shown to react with HLA-A in wild-type A-431 cells in Western blot Loss of signal was observed when HLA-A knockout cell line **ab261894** (knockout cell lysate ab261703) was used. Wild-type A-431 and HLA-A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab52922** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed 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 hour at room temperature before imaging.



Next Generation Sequencing - Human HLA-A knockout A-431 cell lysate (ab261703)

X = 17 bp deletion

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