abcam

Product datasheet

Human ACTA2 knockout HeLa cell lysate ab264499

4 Images

Overview

Product name	Human ACTA2 knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
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

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

Components	1 kit
ab280497 - Human ACTA2 knockout HeLa cell lysate	1 x 100µg
ab269597 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

Target

Function Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.

Involvement in disease Defects in ACTA2 are the cause of aortic aneurysm familial thoracic type 6 (AAT6) [MIM:611788]. AATs are characterized by permanent dilation of the thoracic aorta usually due to degenerative changes in the aortic wall. They are primarily associated with a characteristic histologic appearance known as 'medial necrosis' or 'Erdheim cystic medial necrosis' in which there is degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, and an accumulation of basophilic ground substance.

Sequence similarities Belongs to the actin family.

Cellular localization Cytoplasm > cytoskeleton.

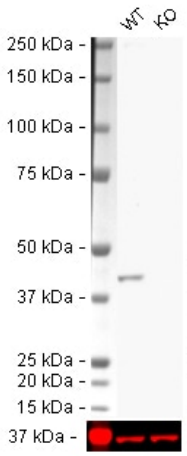
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab264499 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: 42 kDa.

Images

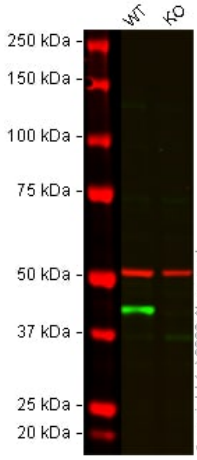


Western blot - Human ACTA2 knockout HeLa cell lysate (ab264499)

Lane 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

ab203696 was shown to react with alpha smooth muscle Actin (HRP) in wild-type HeLa cells in western blot. Loss of signal was observed when ACTA2 knockout cell line **ab264014** (knockout cell lysate ab264499) was used. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab203696** overnight at 4°C at a 1 in 5000 dilution and **ab184095** (Mouse Anti-GAPDH antibody [mAbcam 9484] - Alexa Fluor® 680) at a 1 in 1000 dilution. Blots were developed with Optiblot ECL reagent (**ab133456**) and imaged.



Western blot - Human ACTA2 knockout HeLa cell lysate (ab264499)

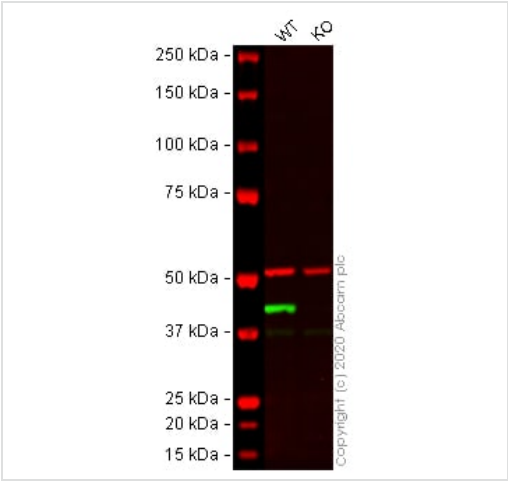
Lane 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lanes 1 - 2: Merged signal (red and green). Green - **ab150301** observed at 42 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab150301 was shown to react with alpha smooth muscle Actin in wild-type HeLa cells in western blot Loss of signal was observed when ACTA2 knockout cell line **ab264014** (knockout cell lysate ab264499) was used. Wild-type HeLa and ACTA2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab150301** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 130 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 hour at room temperature before imaging.



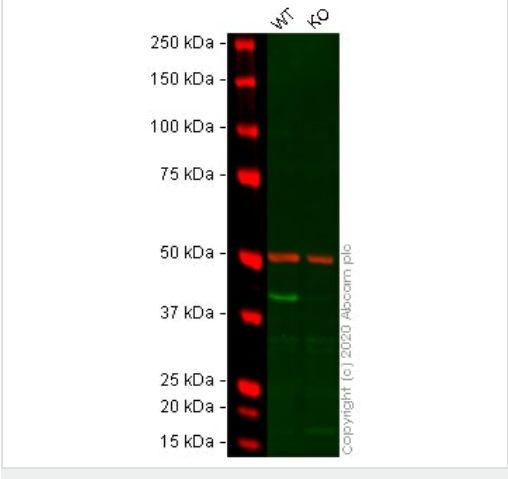
Western blot - Human ACTA2 knockout HeLa cell lysate (ab264499)

Lane 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lanes 1 - 2: Merged signal (red and green). Green - **ab124964** observed at 42 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab124964 was shown to react with alpha smooth muscle Actin in wild-type HeLa cells in western blot Loss of signal was observed when ACTA2 knockout cell line **ab264014** (knockout cell lysate ab264499) was used. Wild-type HeLa and ACTA2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab124964** 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 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 hour at room temperature before imaging.



Western blot - Human ACTA2 knockout HeLa cell lysate (ab264499)

Lane 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lanes 1 - 2: Merged signal (red and green). Green - **ab7817** observed at 42 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab7817 was shown to react with alpha smooth muscle Actin in wild-type HeLa cells in western blot Loss of signal was observed when ACTA2 knockout cell line **ab264014** (knockout cell lysate ab264499) was used. Wild-type HeLa and ACTA2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab7817** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 131.58 dilution and a 1 in 20000 dilution

respectively. Blots were incubated 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

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