# abcam

## Product datasheet

## Human ACTA2 knockout HeLa cell lysate ab264499

## 画像数 4

#### 製品の概要

製品名 Human ACTA2 knockout HeLa cell lysate

製品の概要 Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell LineHeLaOrganismHumanPassage number<20</th>

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.

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

#### 製品の特性

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#### 保存方法

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

内容	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

#### ターゲット情報

機能 Actins are highly conserved proteins that are involved in various types of cell motility and are

ubiquitously expressed in all eukaryotic cells.

**関連疾患** 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.

**配列類似性** Belongs to the actin family.

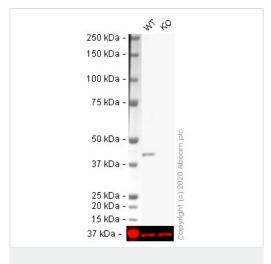
**細胞内局在** Cytoplasm > cytoskeleton.

#### アプリケーション

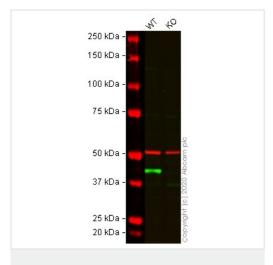
**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab264499の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 42 kDa.

#### 画像



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



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

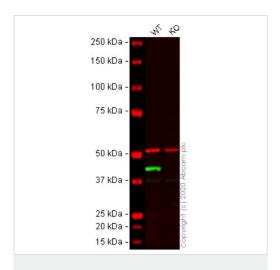
<u>ab203696</u> was shown to react with alpha smooth muscle Actin (HRP) in wild-type HeLa cells in western blot. Loss of signal was observed when ACTA2 knockout cell line <u>ab264014</u> (knockout cell lysate ab264499) was used. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before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

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

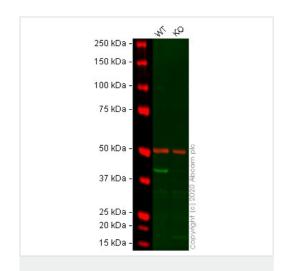
Lane 2: ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lanes 1 - 2:** Merged signal (red and green). Green - <u>ab150301</u> observed at 42 kDa. Red - loading control, <u>ab7291</u> (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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®



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



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

680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

**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 - <u>ab124964</u> observed at 42 kDa. Red - loading control, <u>ab7291</u> (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.

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 - <u>ab7817</u> observed at 42 kDa. Red - loading control, <u>ab52866</u> (Rabbit antialpha 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 (<u>ab216772</u>) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (<u>ab216777</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging

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