abcam

Product datasheet

Human ACTA2 knockout HeLa cell line ab264014

1 References　画像数 12

製品の概要

製品名	Human ACTA2 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
アプリケーション	**適用あり:** ICC, WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab271142**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required. Cells should be passaged when they have achieved 80-90% confluence.

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**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

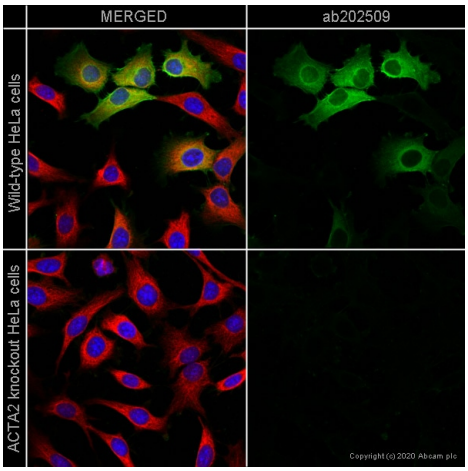
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab264014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。

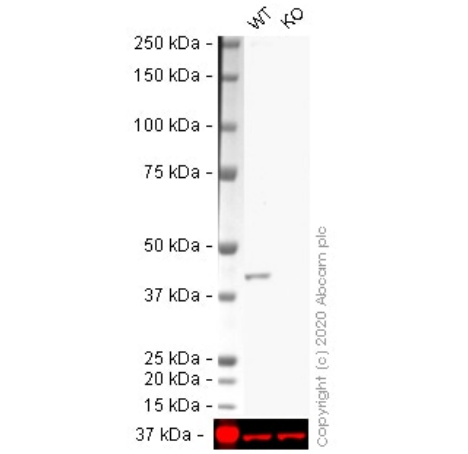
アプリケーション	Abreviews	特記事項
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.

画像



Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab202509 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab202509** at 1/200 dilution and **ab195884** (Rat monoclonal to Tubulin - Alexa Fluor® 647) at 1/100 dilution overnight at 4°C. Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human ACTA2 knockout HeLa cell line (ab264014)

All lanes : HRP Anti-alpha smooth muscle Actin antibody [1A4] (**ab203696**) at 1/5000 dilution

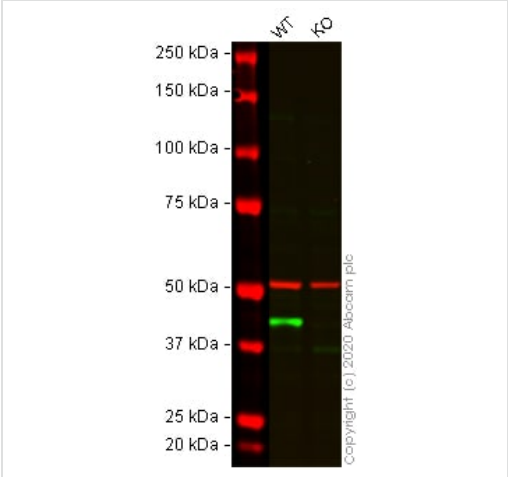
Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Exposure time: 20 seconds

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 line (ab264014)

All lanes : Anti-alpha smooth muscle Actin antibody [SP171] (**ab150301**) at 1/130 dilution

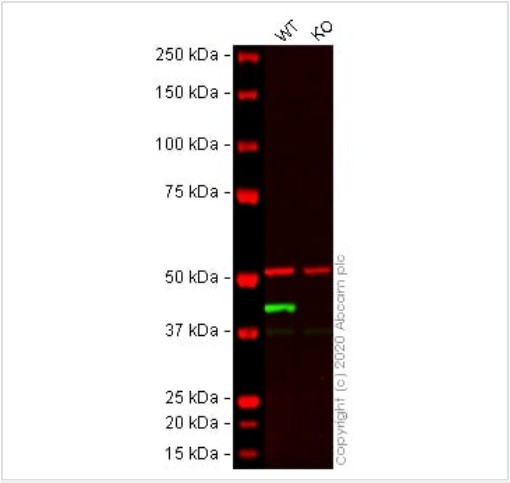
Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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 line (ab264014)

All lanes : Anti-alpha smooth muscle Actin antibody [EPR5368] (**ab124964**) at 1/10000 dilution

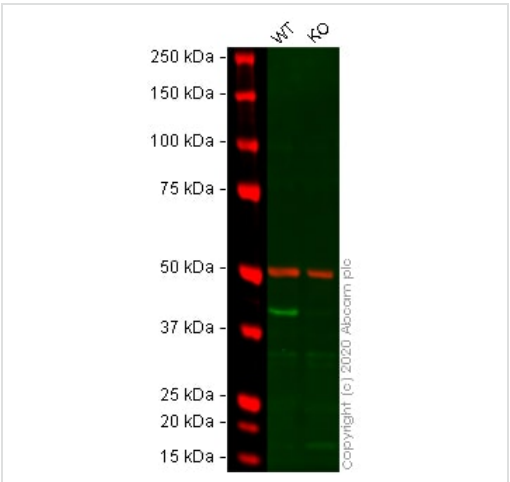
Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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 line (ab264014)

All lanes : Anti-alpha smooth muscle Actin antibody [1A4] (**ab7817**) at 1/131.58 dilution

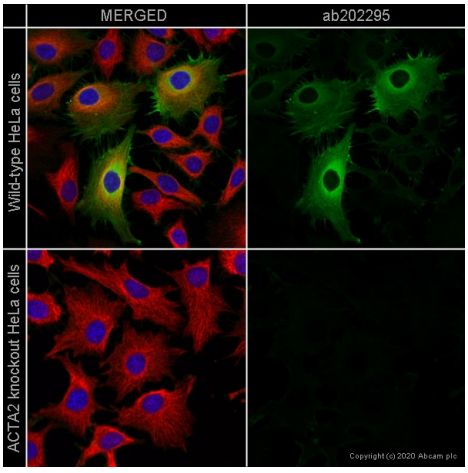
Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Performed under reducing conditions.

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.

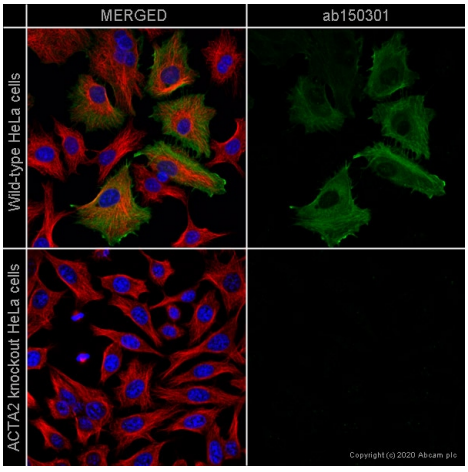
ab7817 was shown to react with alpha smooth muscle Actin in

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



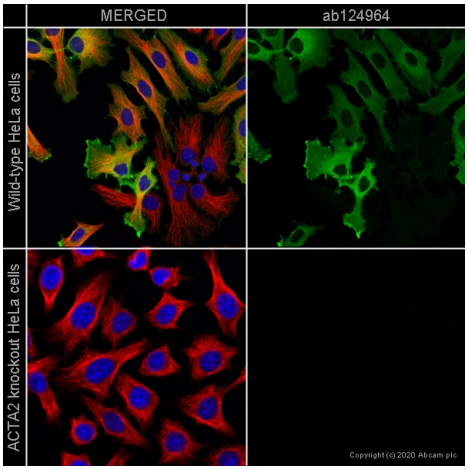
Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab202295 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab202295** at 1/500 dilution and **ab195884** (Rat monoclonal to Tubulin - Alexa Fluor® 647) at 1/100 dilution overnight at 4°C. Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



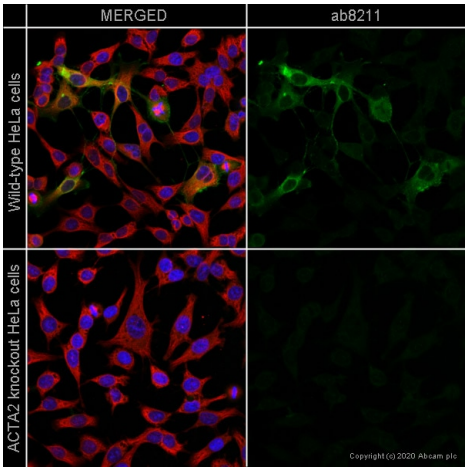
Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab150301 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab150301** at 5 ug/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 ug/ml (shown in green) and a goat secondary antibody to mouse IgG (AAlexa Fluor® 594) (**ab150120**) at 2 ug/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



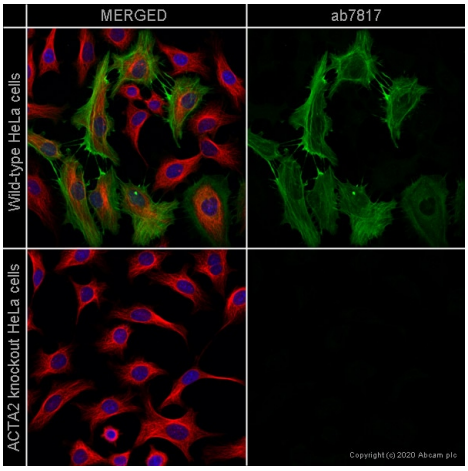
Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab124964 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab124964** at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4^{o}C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 ug/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 ug/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



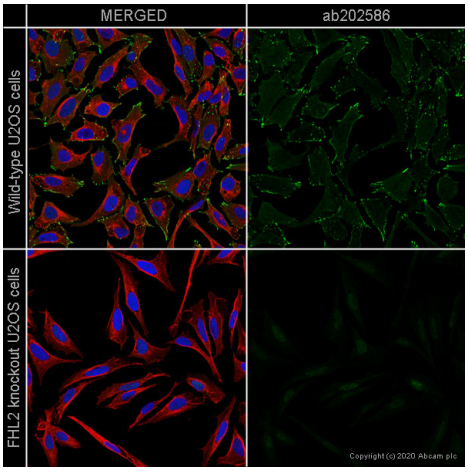
Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab8211 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab8211** at 5 ug/ml concentration and **ab195884** (Rat monoclonal to Tubulin - Alexa Fluor® 647) at 1/100 dilution overnight at 4^{o}C. Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

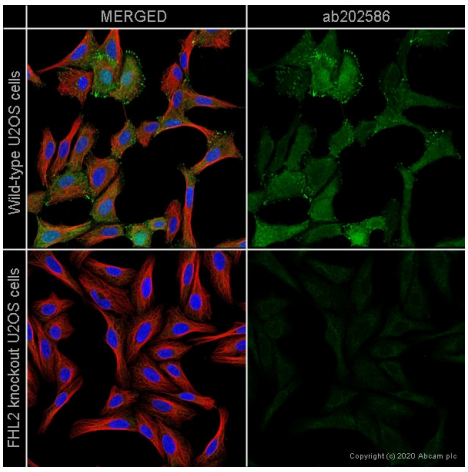
ab7817 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab7817** at 5ug/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4^{O}C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 ug/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 ug/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab202586 staining FHL2 in wild-type U-2 OS cells (top panel) and FHL2 knockout U-2 OS cells (ab264014) (bottom panel). The cells were fixed with 4% PFA (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab202586** at 1/100 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human ACTA2 knockout HeLa cell line (ab264014)

ab202586 staining FHL2 in wild-type U-2 OS cells (top panel) and FHL2 knockout U-2 OS cells (ab264014) (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab202586** at 1/100 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.co.jp/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors