abcam

Product datasheet

Anti-FHL2 antibody [EPR17860-20] ab202584

KO 評価済 リコンビナント RabMAb

4 References 画像数 11

製品の概要

製品名	Anti-FHL2 antibody [EPR17860-20]
製品の詳細	Rabbit monoclonal [EPR17860-20] to FHL2
由来種	Rabbit
アプリケーション	**適用あり:** WB, ICC/IF, IP
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: U-2 OS, K526, HeLa, SW480, PC-12 and HT1080 whole cell lysates; Human fetal heart lysate; Mouse and rat heart lysates. ICC/IF: A-673 and NIH/3T3 cells. IP: SW480 whole cell lysate. ICC/IF: U-2 OS cells.
特記事項	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR17860-20

アイソタイプ	IgG

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ab202584の使用に適用されます**

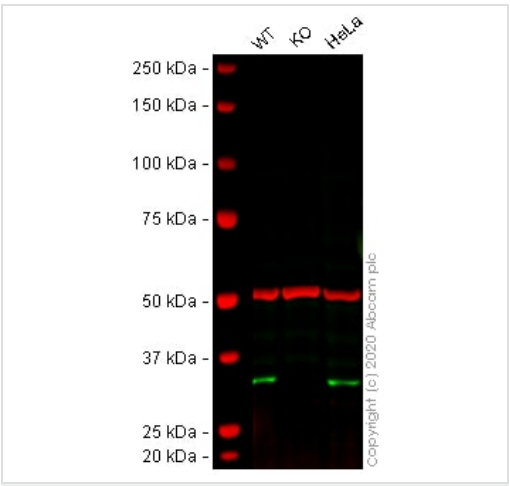
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 32 kDa (predicted molecular weight: 32 kDa).
ICC/IF		1/400 - 1/200. ab202584 works both with PFA and methanol fixation. Fixation with PFA gives the strongest signal.
IP		1/30.

ターゲット情報

機能	May function as a molecular transmitter linking various signaling pathways to transcriptional regulation. Negatively regulates the transcriptional repressor E4F1 and may function in cell growth.
組織特異性	Expressed in skeletal muscle and heart.
配列類似性	Contains 4 LIM zinc-binding domains.
ドメイン	The third LIM zinc-binding mediates interaction with E4F1.
細胞内局在	Cytoplasm. Nucleus.

画像



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : Wild-type U-2 OS cell lysate
Lane 2 : FHL2 knockout U-2 OS cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

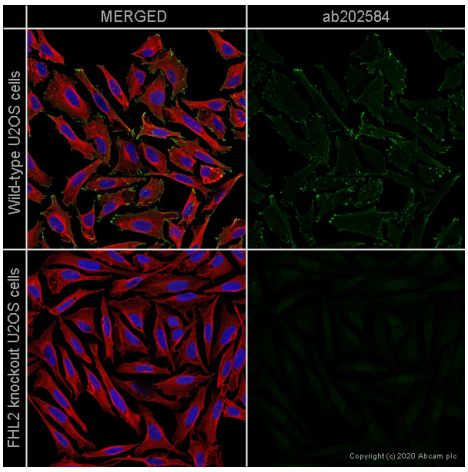
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa
Observed band size: 32 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab202584 observed at 32 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

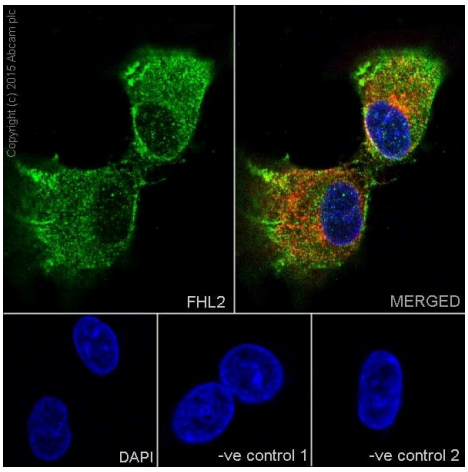
ab202584 was shown to react with FHL2 in wild-type U-2 OS cells in western blot with loss of signal observed in FHL2 knockout sample.Wild-type and FHL2 knockout U-2 OS cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab202584 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 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.



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

ab202584 staining FHL2 in wild-type U2OS cells (top panel) and FHL2 knockout U2OS cells (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 ab202584 at 1/200 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.

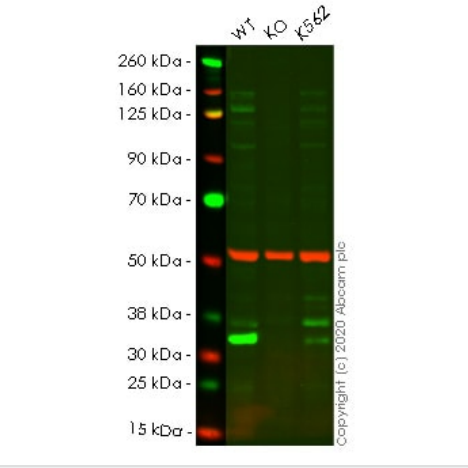
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A-673 (Human muscle Ewing's Sarcoma cell line) cells labeling FHL2 with ab202584 at 1/400 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on A-673 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: ab202584 at 1/400 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : Wild-type HeLa lysate
Lane 2 : FHL2 knockout HeLa lysate
Lane 3 : K562 lysate

Lysates/proteins at 20 µg per lane.

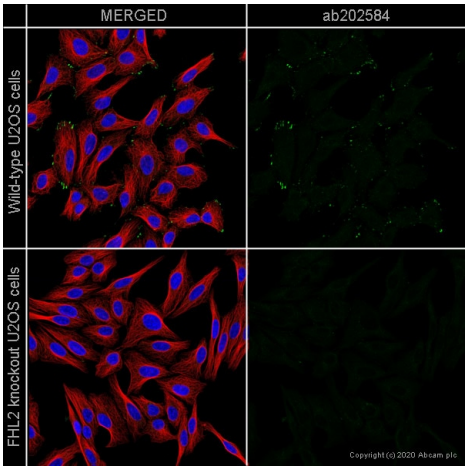
Performed under reducing conditions.

Predicted band size: 32 kDa

Lanes 1-3: Merged signal (red and green). Green - ab202584 observed at 32 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab202584 Recombinant Anti-FHL2 antibody [EPR17860-20] was shown to specifically react with FHL2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265475** (knockout cell lysate **ab257441**) was used. Wild-type and FHL2 knockout samples were subjected to SDS-PAGE. ab202584 and Anti-alpha Tubulin antibody [DM1A] - Loading Control?(**ab7291**) 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® 800CW) preadsorbed (**ab216773**) and

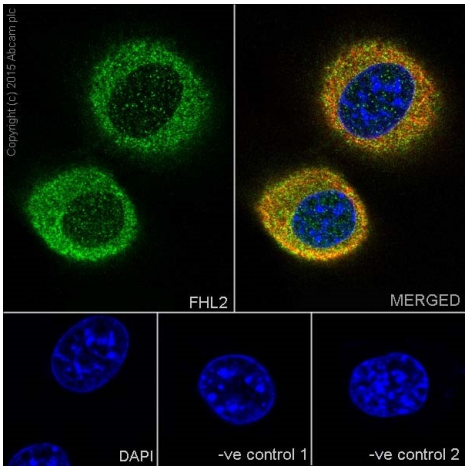
Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

ab202584 staining FHL2 in wild-type U2OS cells (top panel) and FHL2 knockout U2OS cells (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 ab202584 at 1/200 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.

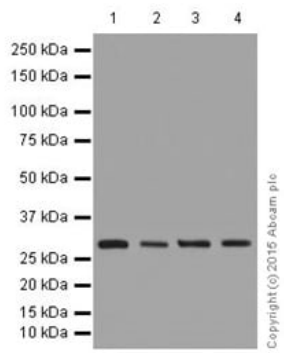
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyro fibroblast cells) cells labeling FHL2 with ab202584 at 1/400 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and weakly nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: ab202584 at 1/400 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1 : SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 3 : HT1080 (Human fibrosarcoma cells) whole cell lysate
Lane 4 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

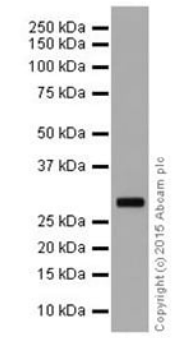
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/10000 dilution + Human fetal heart lysate at 10 µg

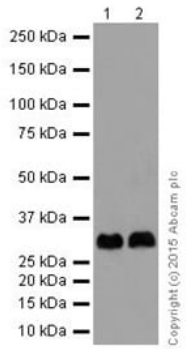
Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-FHL2 antibody [EPR17860-20] (ab202584)

All lanes : Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/2000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Rat heart lysate

Lysates/proteins at 10 µg per lane.

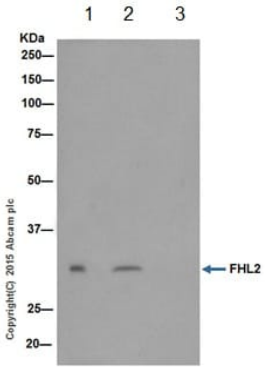
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 32 kDa
Observed band size: 32 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-FHL2 antibody [EPR17860-20] (ab202584)

FHL2 was immunoprecipitated from 1mg of SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate with ab202584 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab202584 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: SW480 whole cell lysate 10 µg (Input). Lane 2: ab202584 IP in SW480 whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab202584 in SW480 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

Anti-FHL2 antibody [EPR17860-20] (ab202584)

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