abcam

Product datasheet

Anti-Caspase-9 antibody [EPR18108] ab185719



ייבעדיו RabMAb

8 References 画像数 9

製品の概要

製品名 Anti-Caspase-9 antibody [EPR18108]

製品の詳細 Rabbit monoclonal [EPR18108] to Caspase-9

由来種 Rabbit

アプリケーション **適用あり:** WB, IP

種交差性 交差種: Mouse, 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: HeLa whole cell lysates untreated and treated with staurosporine 1uM for 4 hours; NIH/3T3

> whole cell lysates untreated and treated with staurosporine1uM for 4 hours; Human fetal brain, fetal heart, fetal kidney and fetal liver and pancreas lysates; Mouse heart and kidney lysates. IP:

HeLa whole cell lysate treated with staurosporine 1uM for 4 hours.

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

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 (glycerin, glycerine), 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 **EPR18108**

アプリケーション

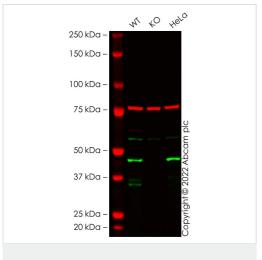
The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab185719の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 46, 37, 35 kDa (predicted molecular weight: 46 kDa).
IP		1/40.

ターゲット情報

機能	Involved in the activation cascade of caspases responsible for apoptosis execution. Binding of caspase-9 to Apaf-1 leads to activation of the protease which then cleaves and activates caspase-3. Proteolytically cleaves poly(ADP-ribose) polymerase (PARP). Isoform 2 lacks activity is an dominant-negative inhibitor of caspase-9.
組織特異性	Ubiquitous, with highest expression in the heart, moderate expression in liver, skeletal muscle, and pancreas. Low levels in all other tissues. Within the heart, specifically expressed in myocytes.
配列類似性	Belongs to the peptidase C14A family. Contains 1 CARD domain.
発生段階	Expressed at low levels in fetal heart, at moderate levels in neonate heart, and at high levels in adult heart.
翻訳後修飾	Cleavages at Asp-315 by granzyme B and at Asp-330 by caspase-3 generate the two active subunits. Caspase-8 and -10 can also be involved in these processing events.

画像



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/1000 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: CASP9 knockout THP-1 cell lysate

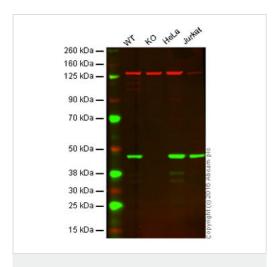
Lane 3: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

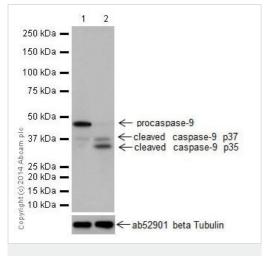
Performed under reducing conditions.

Predicted band size: 46 kDa **Observed band size:** 45 kDa

False colour image of Western blot: Anti-Caspase-9 antibody [EPR18108] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab185719 was shown to bind specifically to Caspase-9. A band was observed at 45 kDa in wild-type THP-1 cell lysates with no signal observed at this size in CASP9 knockout cell line ab276122 (knockout cell lysate ab284219). To generate this image, wild-type and CASP9 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature. washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: Caspase-9 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: Jurkat cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab185719 observed at 46 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab185719 was shown to specifically react with Caspase-9 when Caspase-9 knockout samples were used. Wild-type and Caspase-9 knockout samples were subjected to SDS-PAGE. ab185719 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/10000 dilution

Lane 1 : Untreated HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lane 2: HeLa whole cell lysate treated with staurosporine 1uM for 4 hours

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

Observed band size: 35,37,46 kDa

Exposure time: 10 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/2500 dilution

Lane 1: Untreated NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lane 2: NIH/3T3 whole cell lysate treated with staurosporine 1uM for 4 hours

Lysates/proteins at 10 µg per lane.



All lanes : Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 46 kDa

Observed band size: 37,39,49 kDa

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/1000 dilution

Lane 1: Human fetal brain lysate

Lane 2: Human fetal heart lysate

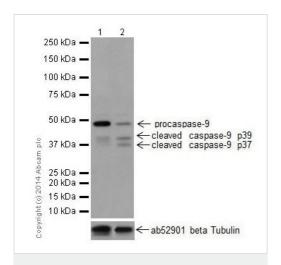
Lane 3: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

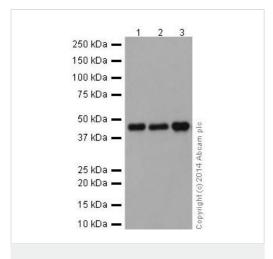
Secondary

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

Predicted band size: 46 kDa **Observed band size:** 46 kDa



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/5000 dilution

Lane 1 : Human pancreas tissue lysate

Lane 2 : Human fetal liver tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

 $\textbf{All lanes:} \ \, \textbf{Anti-Rabbit lgG (HRP), specific to the non-reduced form}$

of IgG at 1/1000 dilution

Predicted band size: 46 kDa **Observed band size:** 46 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Caspase-9 antibody [EPR18108] (ab185719) at 1/1000 dilution

Lane 1 : Mouse heart tissue lysate

Lane 2 : Mouse kidney tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at

1/1000 dilution

Predicted band size: 46 kDa

Observed band size: 37,39,49 kDa

Exposure time: 15 seconds

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)



Western blot - Anti-Caspase-9 antibody [EPR18108] (ab185719)

Blocking/Dilution buffer: 5% NFDM/TBST.

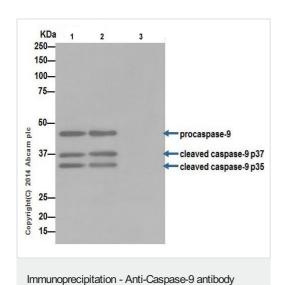
Caspase-9 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate treated with staurosporine 1uM for 4 hours with ab185719 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab185719 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: HeLa whole cell lysate treated with staurosporine 10 μg (Input).

Lane 2: ab185719 IP in HeLa whole cell lysate treated with staurosporine.

Lane 3: Rabbit monoclonal $\lg G$ (<u>ab172730</u>) instead of ab185719 in HeLa whole cell lysate treated with staurosporine.

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



[EPR18108] (ab185719)



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