abcam

Product datasheet

Anti-DR5 antibody [EPR19310] ab199357

敲除验证 重组 RabMAb

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概述

产品名称	Anti-DR5抗体[EPR19310]
描述	兔单克隆抗体[EPR19310] to DR5
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IP
种属反应性	**与反应:** Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Untreated and treated with 0.5ÂµM/ml doxorubicin for 24 hours HCT 116 whole cell lysates; HeLa, HAP1, HepG2 and HT1080 whole cell lysates; Human melanoma lysate. ICC/IF: HT1080 and HCT 116 cells. Flow Cyt (intra): HCT 116 cells. IP: HT-1080 treated with 5ÂµM MG132 for 4 hour whole cell lysate; HCT 116 whole cell lysate.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR19310

同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab199357于以下的经测试应用

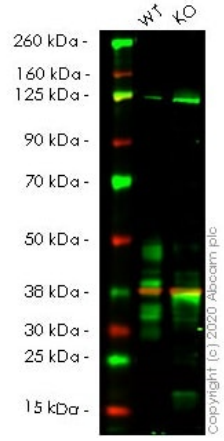
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/70.
WB	★★★★★ (2)	1/1000. Detects a band of approximately 48, 40 kDa (predicted molecular weight: 48 kDa).
ICC/IF		1/100.
IP		1/30.

靶标

功能	Receptor for the cytotoxic ligand TNFSF10/TRAIL. The adapter molecule FADD recruits caspase-8 to the activated receptor. The resulting death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. Promotes the activation of NF-kappa-B. Essential for ER stress-induced apoptosis.
组织特异性	Widely expressed in adult and fetal tissues; very highly expressed in tumor cell lines such as HeLaS3, K-562, HL-60, SW480, A-549 and G-361; highly expressed in heart, peripheral blood lymphocytes, liver, pancreas, spleen, thymus, prostate, ovary, uterus, placenta, testis, esophagus, stomach and throughout the intestinal tract; not detectable in brain.
疾病相关	Squamous cell carcinoma of the head and neck
序列相似性	Contains 1 death domain. Contains 3 TNFR-Cys repeats.
细胞定位	Membrane.

图片



Western blot - Anti-DR5 antibody [EPR19310] (ab199357)

All lanes : Anti-DR5 antibody [EPR19310] (ab199357) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : DR5 knockout HeLa cell lysate

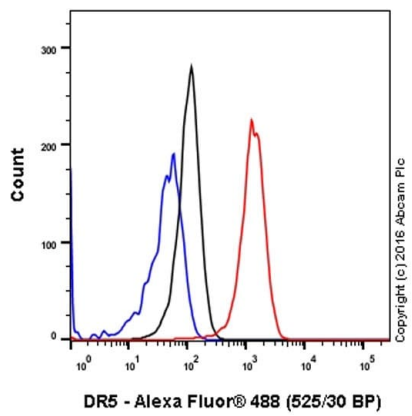
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa
Observed band size: 47 kDa

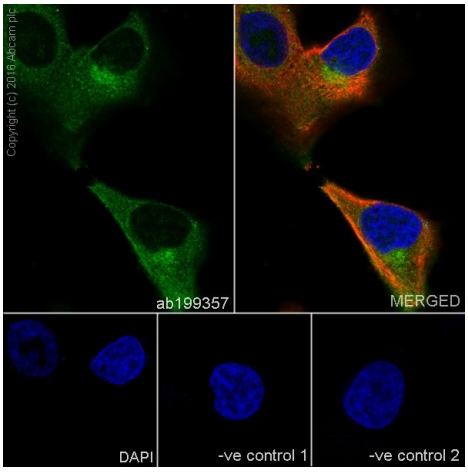
Lanes 1- 2: Merged signal (red and green). Green - ab199357 observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab199357 was shown to react with DR5 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264922** (knockout cell lysate **ab257748**) was used. Wild-type HeLa and DR5 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab199357 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Flow Cytometry (Intracellular) - Anti-DR5 antibody [EPR19310] (ab199357)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HCT 116 (Human colorectal carcinoma cell line) cells labeling DR5with ab199357 at 1/70 dilution (red) compared with aRabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti Rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution was used as the secondary antibody.

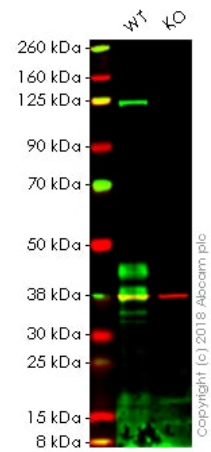


Immunocytochemistry/ Immunofluorescence - Anti-DR5 antibody [EPR19310] (ab199357)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HT1080 (Human fibrosarcoma cell line) cells labeling DR5 with ab199357 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HT1080 cells. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab199357 at 1/100 dilution followed by **ab150120** at 1/1000 dilution.
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Western blot - Anti-DR5 antibody [EPR19310] (ab199357)

All lanes : Anti-DR5 antibody [EPR19310] (ab199357) at 1/1000 dilution

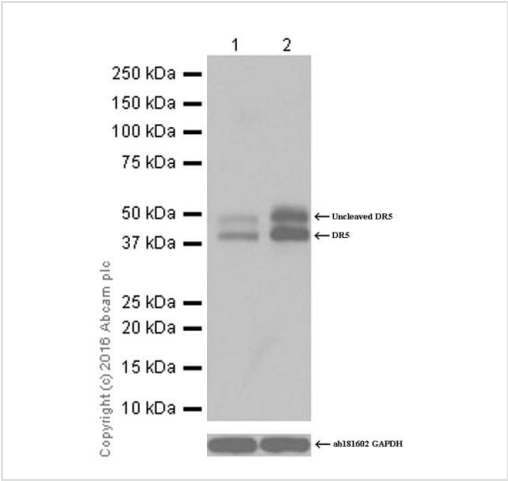
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : TNFRSF10B knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 48 kDa
Observed band size: 47 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab199357 observed at 47 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab199357 was shown to recognize DR5 in wild-type HAP1 cells as signal was lost at the expected MW in TNFRSF10B knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and TNFRSF10B knockout samples were subjected to SDS-PAGE. Ab199357 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-DR5 antibody [EPR19310] (ab199357)

All lanes : Anti-DR5 antibody [EPR19310] (ab199357) at 1/1000 dilution

Lane 1 : Untreated HCT 116 (Human colorectal carcinoma cell line) whole cell lysate
Lane 2 : HCT 116 (Human colorectal carcinoma cell line) treated with 0.5µM/ml doxorubicin for 24 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

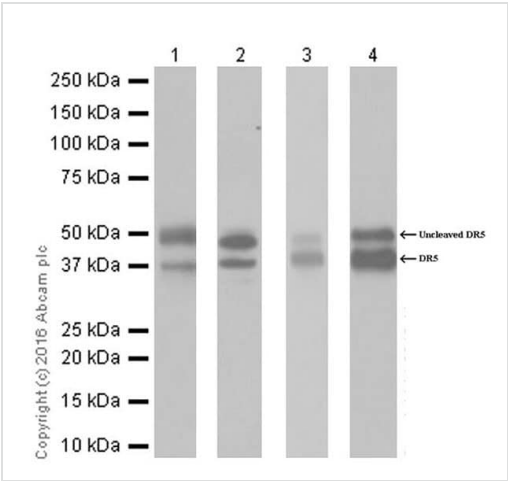
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 48 kDa
Observed band size: 40,48 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Doxorubicin treatment elevated the expression of DR5 (PMID: 12496481; PMID: 11468181; PMID: 11090076). The expression profile is consistent with what has been described in the literature (PMID:20515924; PMID:16297203).



Western blot - Anti-DR5 antibody [EPR19310] (ab199357)

All lanes : Anti-DR5 antibody [EPR19310] (ab199357) at 1/1000 dilution

Lane 1 : Human melanoma lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 4 : HT1080 (Human fibrosarcoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

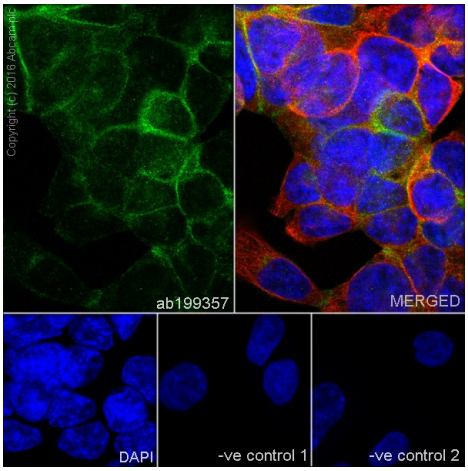
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 48 kDa
Observed band size: 40,48 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-2: 3 minutes; Lane 3: 10 seconds; Lane 4: 8 seconds.

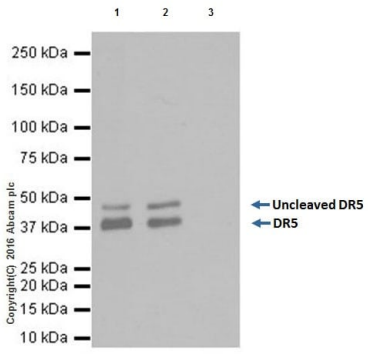
The expression profile is consistent with what has been described in the literature (PMID:20515924; PMID:16297203).



Immunocytochemistry/ Immunofluorescence - Anti-DR5 antibody [EPR19310] (ab199357)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (Human colorectal carcinoma cell line) cells labeling DR5 with ab199357 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on HCT 116 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab199357 at 1/100 dilution followed by **ab150120** at 1/1000 dilution.
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Immunoprecipitation - Anti-DR5 antibody [EPR19310] (ab199357)

DR5 was immunoprecipitated from 0.35mg of HT1080 (Human fibrosarcoma cell line) treated with 5µM MG132 for 4 hour whole cell lysate with ab199357 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab199357 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

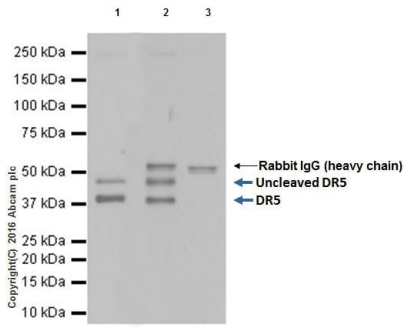
Lane 1: HT1080 treated with 5µM MG132 for 4 hour whole cell lysate, 10µg (Input).

Lane 2: ab199357 IP in HT1080 treated with 5µM MG132 for 4 hour whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab199357 in HT1080 treated with 5µM MG132 for 4 hour whole cell lysate.

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

Exposure time: 10 seconds.



Immunoprecipitation - Anti-DR5 antibody [EPR19310] (ab199357)

DR5 was immunoprecipitated from 0.35mg of HCT 116 (Human colorectal carcinoma cell line) whole cell lysate with ab199357 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab199357 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HCT 116 whole cell lysate, 10µg (Input).

Lane 2: ab199357 IP in HCT 116 whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab199357 in HCT 116 whole cell lysate.

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

Exposure time: 10 seconds.

Anti-DR5 antibody [EPR19310] (ab199357)

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