abcam

Product datasheet

Anti-DR5 antibody [EPR19310] ab199357





★★★★★ 5 Abreviews 12 References 10 Images

Overview

Product name Anti-DR5 antibody [EPR19310]

Description Rabbit monoclonal [EPR19310] to DR5

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Untreated and treated with 0.5µM/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 5µM MG132 for 4

hour whole cell lysate; HCT 116 whole cell lysate.

General notes 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**.

Properties

Form

Storage instructions 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.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR19310

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab199357 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/70.
WB	*** <u>*</u>	1/1000. Detects a band of approximately 48, 40 kDa (predicted molecular weight: 48 kDa).
ICC/IF		1/100.
IP		1/30.

Target

Function 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.

Tissue specificity 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.

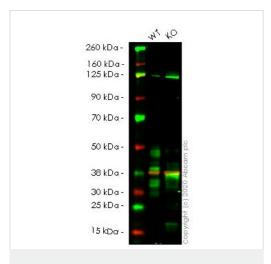
Involvement in disease Squamous cell carcinoma of the head and neck

Sequence similarities Contains 1 death domain.

Contains 3 TNFR-Cys repeats.

Cellular localization Membrane.

Images



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

Lane 1: Wild-type HeLa cell lysate

Lane 2: DR5 knockout HeLa cell lysate

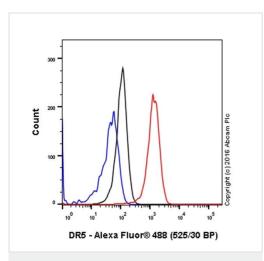
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

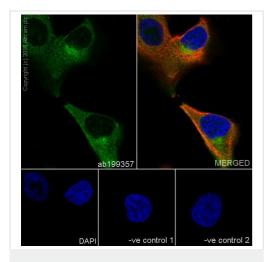
Lanes 1-2: Merged signal (red and green). Green - ab199357 observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

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 lgG,monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti Rabbit lgG (Alexa Fluorr® 488) at 1/2000 dilution was used as the secondary antibody.



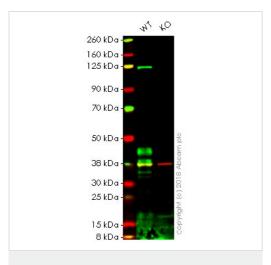
Immunocytochemistry/ Immunofluorescence - Anti-DR5 antibody [EPR19310] (ab199357)

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 lgG 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: $\underline{ab7291}$ at 1/1000 dilution followed by $\underline{ab150077}$ at 1/1000 dilution.



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

Lane 1: Wild-type HAP1 whole cell lysate

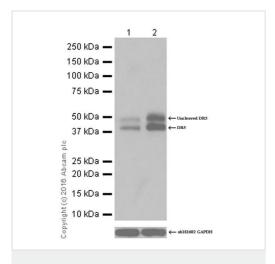
Lane 2: TNFRSF10B knockout HAP1 whole cell lysate

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, <u>ab9484</u>, 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)

Lane 1 : Untreated HCT 116 (Human colorectal carcinoma cell line) whole cell lysate

Lane 2 : HCT 116 (Human colorectal carcinoma cell line) treated with $0.5\mu M/ml$ doxorubicin for 24 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

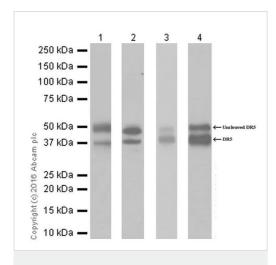
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

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)

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 $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

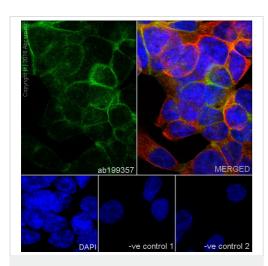
Predicted band size: 48 kDa **Observed band size:** 40,48 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

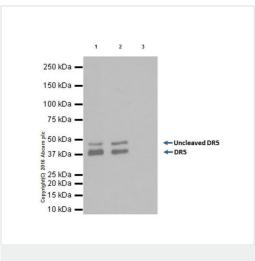
Exposure time: Lane 1-2: 3 minutes; Lane 3: 10 seconds; Lane 4: 8

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)



Immunoprecipitation - 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 $\underline{ab150120}$ at 1/1000 dilution.

-ve control 2: <u>ab7291</u> at 1/1000 dilution followed by <u>ab150077</u> at 1/1000 dilution.

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.

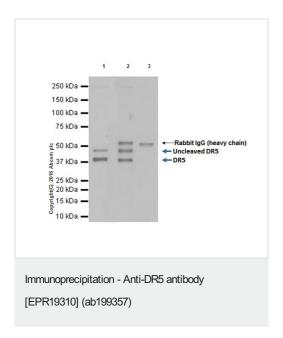
Lane 1: HT1080 treated with $5\mu M$ MG132 for 4 hour whole cell lysate, $10\mu g$ (Input).

Lane 2: ab199357 IP in HT1080 treated with $5\mu M$ MG132 for 4 hour whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A] - Isotype Control ($\underline{ab172730}$) 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.



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 lgG,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.



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.com/abpromise or contact our technical team.

Terms and conditions

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