abcam

Product datasheet

Anti-PMS2 antibody [EPR3947] - BSA and Azide free ab214442



ייבעבער RabMAb

画像数 12 4 References

製品の概要

特記事項

製品名 Anti-PMS2 antibody [EPR3947] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR3947] to PMS2 - BSA and Azide free

由来種 Rabbit

アプリケーション 適用あり: ICC/IF, Flow Cyt (Intra), IHC-P, WB, IP

交差種: Human 種交差性

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Hap1 and HeLa cell lysates. IP: HeLa whole cell lysate.

ab214442 is the carrier-free version of ab110638.

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

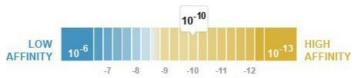
製品の特性

1

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

解離定数(K_D 値) $K_D = 1.50 \times 10^{-10} M$



Learn more about K_D

バッファー pH: 7.20

Constituent: 100% PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR3947

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab214442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/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 96 kDa).
IP		Use at an assay dependent concentration.

ターゲット情報

機能

Component of the post-replicative DNA mismatch repair system (MMR). Heterodimerizes with MLH1 to form MutL alpha. DNA repair is initiated by MutS alpha (MSH2-MSH6) or MutS beta (MSH2-MSH6) binding to a dsDNA mismatch, then MutL alpha is recruited to the heteroduplex. Assembly of the MutL-MutS-heteroduplex ternary complex in presence of RFC and PCNA is sufficient to activate endonuclease activity of PMS2. It introduces single-strand breaks near the mismatch and thus generates new entry points for the exonuclease EXO1 to degrade the strand

containing the mismatch. DNA methylation would prevent cleavage and therefore assure that only the newly mutated DNA strand is going to be corrected. MulL alpha (MLH1-PMS2) interacts physically with the clamp loader subunits of DNA polymerase III, suggesting that it may play a role to recruit the DNA polymerase III to the site of the MMR. Also implicated in DNA damage signaling, a process which induces cell cycle arrest and can lead to apoptosis in case of major DNA damages.

関連疾患

Defects in PMS2 are the cause of hereditary non-polyposis colorectal cancer type 4 (HNPCC4) [MIM:600259]. Mutations in more than one gene locus can be involved alone or in combination in the production of the HNPCC phenotype (also called Lynch syndrome). Most families with clinically recognized HNPCC have mutations in either MLH1 or MSH2 genes. HNPCC is an autosomal, dominantly inherited disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early onset colorectal carcinoma (CRC) and extra-colonic cancers of the gastrointestinal, urological and female reproductive tracts. HNPCC is reported to be the most common form of inherited colorectal cancer in the Western world, and accounts for 15% of all colon cancers. Cancers in HNPCC originate within benign neoplastic polyps termed adenomas. Clinically, HNPCC is often divided into two subgroups. Type I: hereditary predisposition to colorectal cancer, a young age of onset, and carcinoma observed in the proximal colon. Type II: patients have an increased risk for cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx in addition to the colon. Diagnosis of classical HNPCC is based on the Amsterdam criteria: 3 or more relatives affected by colorectal cancer, one a first degree relative of the other two; 2 or more generation affected; 1 or more colorectal cancers presenting before 50 years of age; exclusion of hereditary polyposis syndromes. The term 'suspected HNPCC' or 'incomplete HNPCC' can be used to describe families who do not or only partially fulfill the Amsterdam criteria, but in whom a genetic basis for colon cancer is strongly suspected.

Defects in PMS2 are a cause of mismatch repair cancer syndrome (MMRCS) [MIM:276300]; also known as Turcot syndrome or brain tumor-polyposis syndrome 1 (BTPS1). MMRCS is an autosomal dominant disorder characterized by malignant tumors of the brain associated with multiple colorectal adenomas. Skin features include sebaceous cysts, hyperpigmented and cafe au lait spots.

Belongs to the DNA mismatch repair mutL/hexB family.

Nucleus.

配列類似性

細胞内局在



Western blot - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)



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

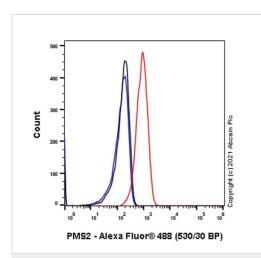
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 96 kDa

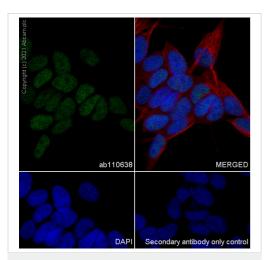
This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.



Flow Cytometry (Intracellular) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442) This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling PMS2 with Purified ab214442 at 1:160 dilution (10 μ g/ml) (Red). Cells were fixed with 4%

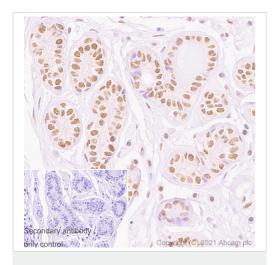
Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150081**) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.

Immunocytochemistry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells labeling PMS2 with Purified ab214442 at 1:200 dilution (8 μg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μg/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2 μg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

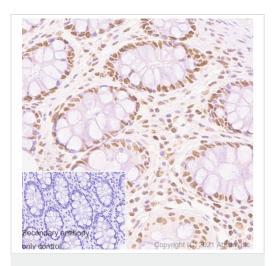


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PMS2 antibody

[EPR3947] - BSA and Azide free (ab214442)

This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast tissue sections labeling PMS2 with Purified ab214442 at 1:600 (2.71 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

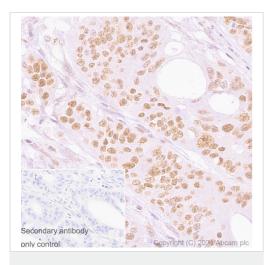


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PMS2 antibody

[EPR3947] - BSA and Azide free (ab214442)

This data was developed using ab214442, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue sections labeling PMS2 with Purified ab214442 at 1:600 (2.71 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

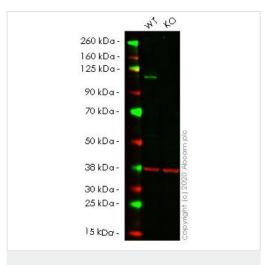


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PMS2 antibody

[EPR3947] - BSA and Azide free (ab214442)

This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon carcinoma tissue sections labeling PMS2 with Purified ab214442 at 1:600 (2.71 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

All lanes : Anti-PMS2 antibody [EPR3947] (ab110638) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: PMS2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

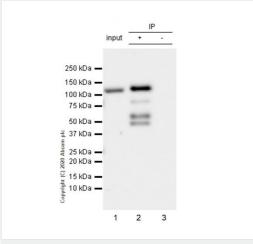
Performed under reducing conditions.

Predicted band size: 96 kDa **Observed band size:** 120 kDa

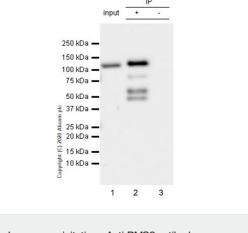
This data was developed using the same antibody clone in a different buffer formulation (<u>ab110638</u>).

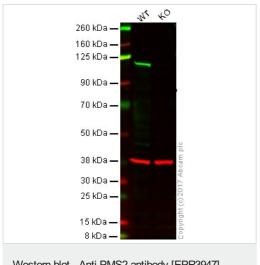
Lanes 1-2: Merged signal (red and green). Green - <u>ab110638</u> observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab110638 was shown to react with PMS2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261776 (knockout cell lysate ab257142) was used. Wild-type HeLa and PMS2 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. ab110638 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.



Immunoprecipitation - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)





Western blot - Anti-PMS2 antibody [EPR3947] -BSA and Azide free (ab214442)

This data was developed using ab110638, the same antibody clone in a different buffer formulation.

Purified ab110638 at 1/50 dilution (2µg) immunoprecipitating PMS2 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab110638 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab110638 in HeLa whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/5000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 110 kDa

Lower bands are degradation bands and fresh lysate is recommended.

All lanes: Anti-PMS2 antibody [EPR3947] (ab110638) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: PMS2 knockout HAP1 whole cell lysate

Lysates/proteins at 30 µg per lane.

Predicted band size: 96 kDa

This WB data was generated using the same anti-PMS2 antibody clone, EPR3947, in a different buffer formulation (cat# ab110638).

Lanes 1-2: Merged signal (red and green). Green - ab110638 observed at 120 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab110638 was shown to specifically react with PMS2 in wild-type HAP1 cells. No band was observed when PMS2 knockout samples were used. Wild-type and PMS2 knockout samples were subjected to SDS-PAGE. Ab110638 and ab8245 (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.

250 kDa —
150 kDa —
150 kDa —
150 kDa —
75 kDa —
250 kDa

Immunoprecipitation - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442) This data was developed using <u>ab110638</u>, the same antibody clone in a different buffer formulation.

Purified $\underline{ab110638}$ at 1/50 dilution (2 μ g) immunoprecipitating PMS2 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab110638 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab110638</u> in HeLa whole cell lysate.

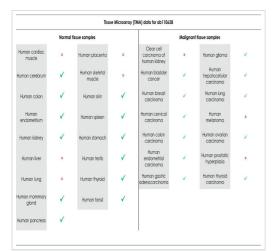
VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/5000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

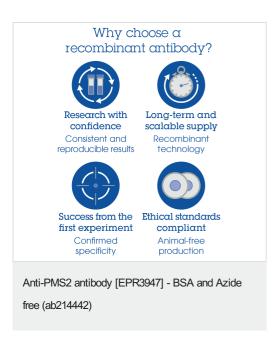
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 110 kDa

Lysate were made freshly and used in IP test immediately to minimize protein degradation. Incubation time was 2h.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442) Tissue Microarrays stained for "Anti-PMS2 antibody [EPR3947]" using "ab110638" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab110638 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



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