abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

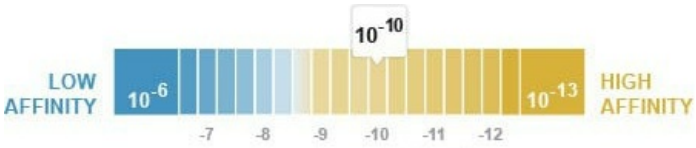
4 References 12 Images

Overview

Product name	Anti-PMS2 antibody [EPR3947] - BSA and Azide free
Description	Rabbit monoclonal [EPR3947] to PMS2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), IHC-P, WB, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Hap1 and HeLa cell lysates. IP: HeLa whole cell lysate.
General notes	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 cell-based 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 1.50 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3947
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214442 in the following tested applications.

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

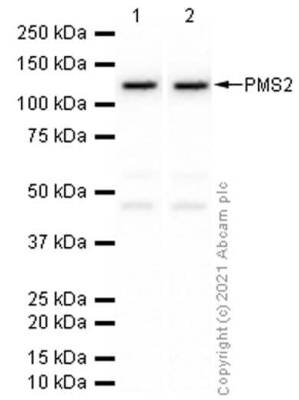
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated 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.

Target

Function 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. MutL 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.
Involvement in disease	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.
Sequence similarities	Belongs to the DNA mismatch repair mutL/hexB family.
Cellular localization	Nucleus.

Images



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

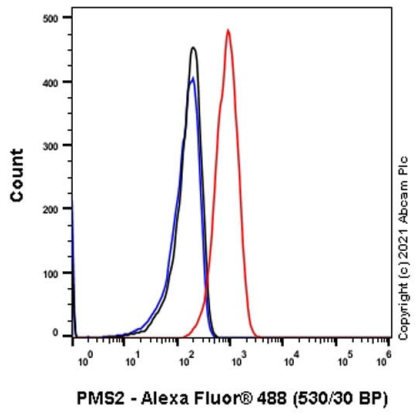
All lanes : Anti-PMS2 antibody [EPR3947] (**ab110638**) at 1/10000 dilution (Purified)

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) (**ab97051**) at 1/20000 dilution

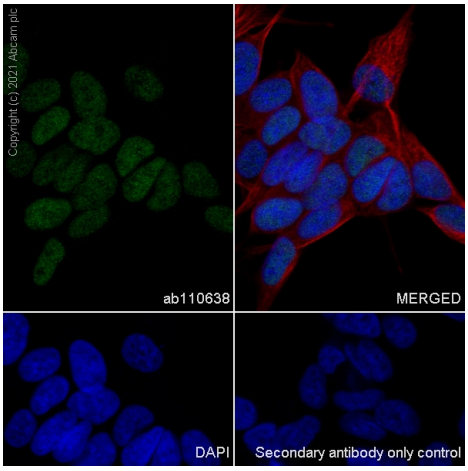
Predicted band size: 96 kDa

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



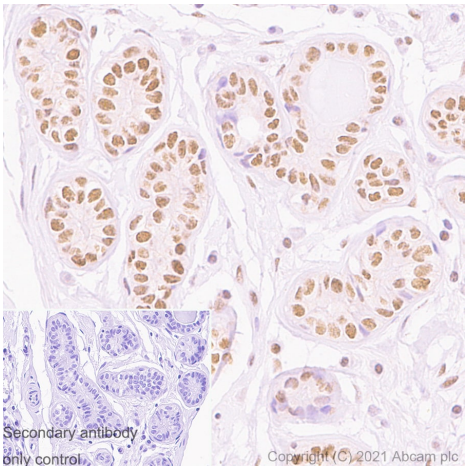
Flow Cytometry (Intracellular) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

This data was developed using **ab110638**, 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 µg/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).



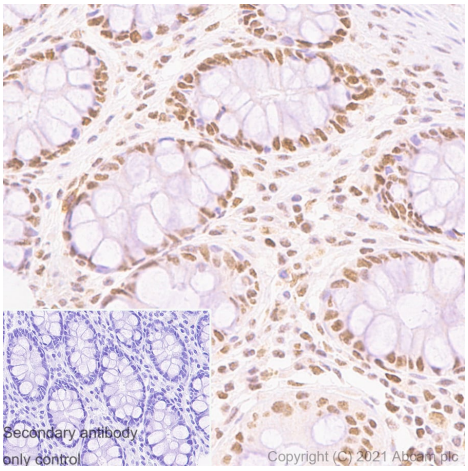
Immunocytochemistry/ Immunofluorescence - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

This data was developed using **ab110638**, 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 IgG (Alexa Fluor® 488, **ab150077**) 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.



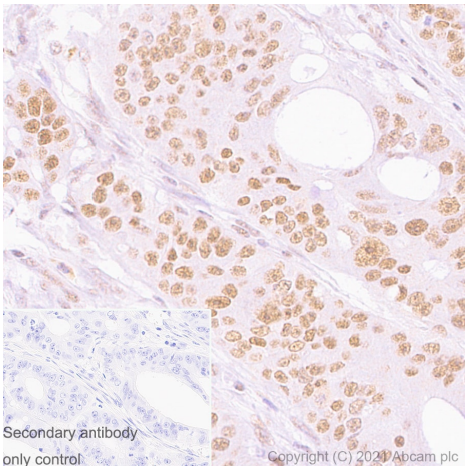
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

This data was developed using **ab110638**, 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.



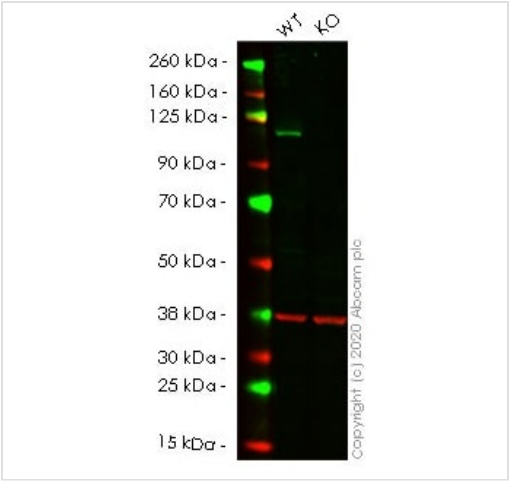
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

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 paraffin-embedded sections) - Anti-PMS2 antibody [EPR3947] - BSA and Azide free (ab214442)

This data was developed using **ab110638**, 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.

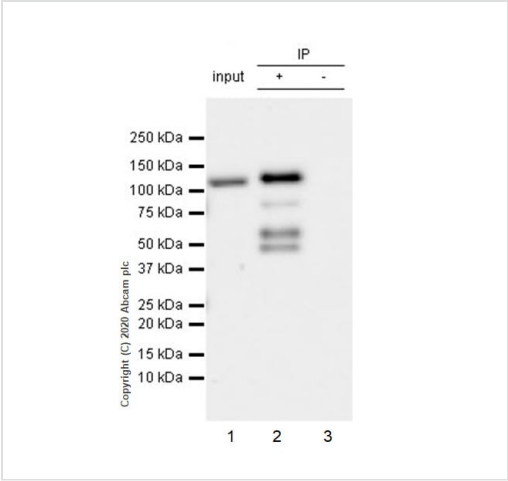
Performed under reducing conditions.

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

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

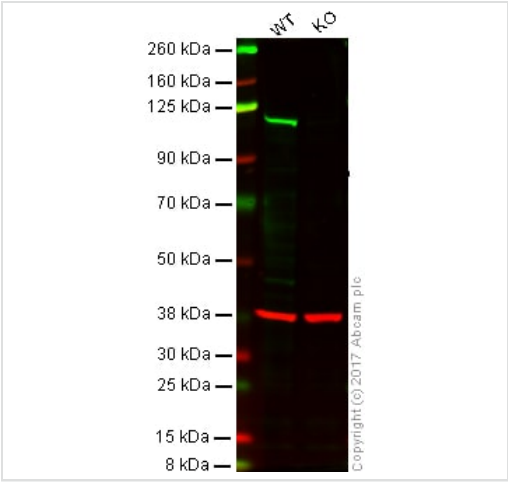
Lanes 1- 2: Merged signal (red and green). Green - **ab110638** observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

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)

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.



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 HAP1 whole cell lysate
Lane 2 : PMS2 knockout HAP1 whole cell lysate

Lysates/proteins at 30 µg per lane.

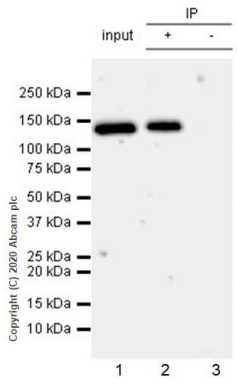
Predicted band size: 96 kDa

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

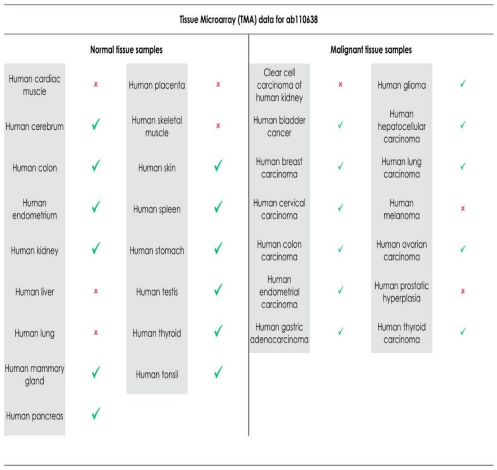
37 kDa.

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.



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

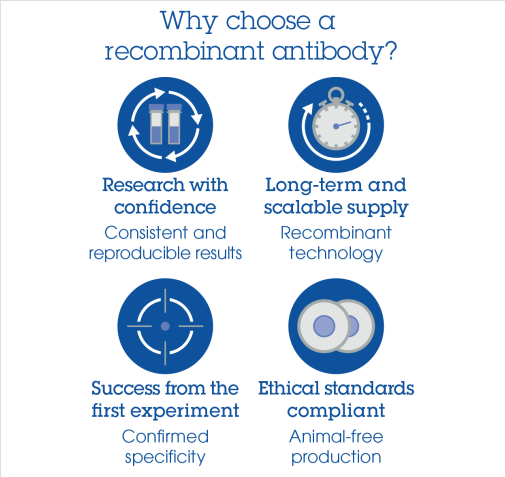
This data was developed using **ab110638**, the same antibody clone in a different buffer formulation.
Purified **ab110638** at 1/50 dilution (2µg) immunoprecipitating PMS2 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): **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
Lysate were made freshly and used in IP test immediately to minimize protein degradation. Incubation time was 2h.


Tissue Microarray (TMA) data for ab110638

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	x	Clear cell carcinoma of human kidney	x	Human glioma	✓
Human cerebrum	✓	Human skeletal muscle	x	Human bladder cancer	✓	Human hepatocellular carcinoma	✓
Human colon	✓	Human skin	✓	Human breast carcinoma	✓	Human lung carcinoma	✓
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	✓	Human melanoma	x
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓	Human ovarian carcinoma	✓
Human liver	x	Human testis	✓	Human endometrial carcinoma	✓	Human prostatic hyperplasia	x
Human lung	x	Human thyroid	✓	Human gastric adenocarcinoma	✓	Human thyroid carcinoma	✓
Human mammary gland	✓	Human tonsil	✓				
Human pancreas	✓						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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.



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

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