abcam

Product datasheet

Anti-PRAME antibody [EPR20330] - BSA and Azide free ab232571

Recombinant RabMAb

15 Images

Overview

Product name	Anti-PRAME antibody [EPR20330] - BSA and Azide free
Description	Rabbit monoclonal [EPR20330] to PRAME - BSA and Azide free
Host species	Rabbit
Specificity	PRAME is expressed in malignant cells, including leukaemias, Hodgkin's lymphoma, breast cancer, and primary and metastatic melanomas.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MeWo and A-375 whole cell lysates; Human ovary cancer and testis lysates. IHC-P: Human melanoma, breast carcinoma and human testis tissue. ICC/IF: MeWo and A-375 cells. Flow Cyt (intra): MeWo cells, K562 cells. IP: MeWo whole cell lysate.
General notes	ab232571 is the carrier-free version of **ab219650**. 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.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20330
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232571 in the following tested applications.

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

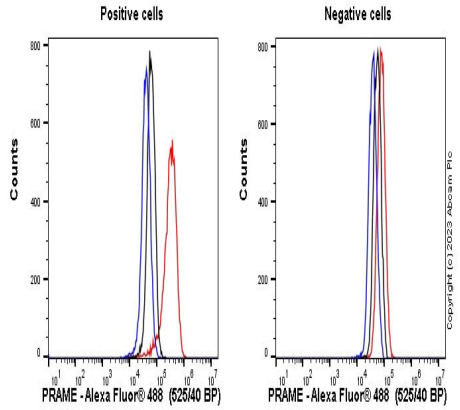
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 57 kDa (predicted molecular weight: 57 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Recommend **ab219650** incubation at +4°C overnight.

Target

Function	Functions as a transcriptional repressor, inhibiting the signaling of retinoic acid through the retinoic acid receptors RARA, RARB and RARG. Prevents retinoic acid-induced cell proliferation arrest, differentiation and apoptosis.
Tissue specificity	Expressed in testis. Detected in samples of kidney, brain and skin.
Sequence similarities	Belongs to the PRAME family. Contains 4 LRR (leucine-rich) repeats.

Cellular localization Nucleus. Cell membrane.

Images



Flow Cytometry (Intracellular) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

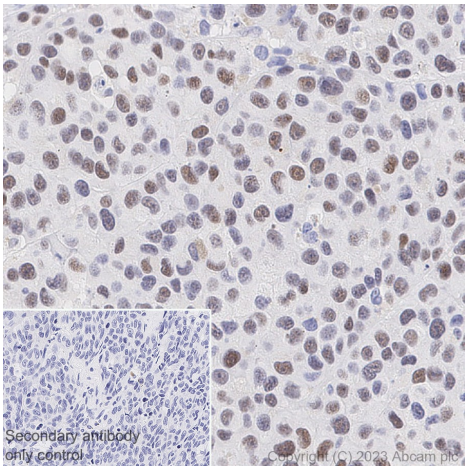
Flow cytometry overlay histogram showing left K562 positive cells and right negative HEK293 stained with **ab219650** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1 % PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab219650**) (1x 10^6 in 100µl at 0.008µg/ml (1/267500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in K562 Fixed with 80% methanol (5 min) / permeabilised with 0.1 % PBS-Triton X-100 for 15 min under the same conditions.



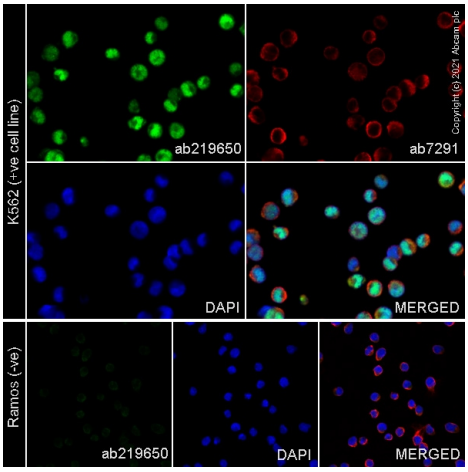
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Nuclear staining on human melanoma. The section was incubated with **ab219650** for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody.



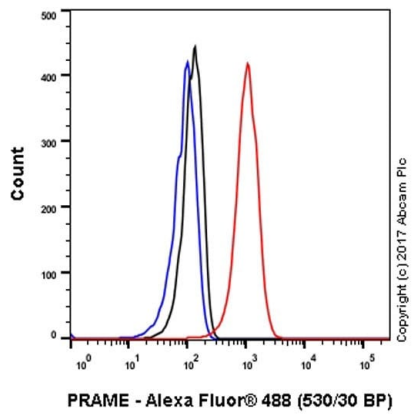
Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

ab219650 staining PRAME in K562 cells, with negative expression in Ramos cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised with 0.1% Triton x-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab219650** at 1 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

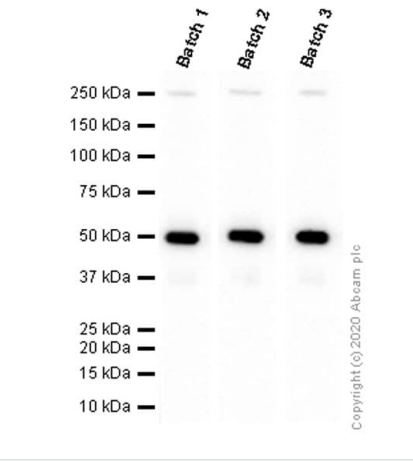
This product also work with 100% methanol (5 min) fixation under the same testing conditions.



Flow Cytometry (Intracellular) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

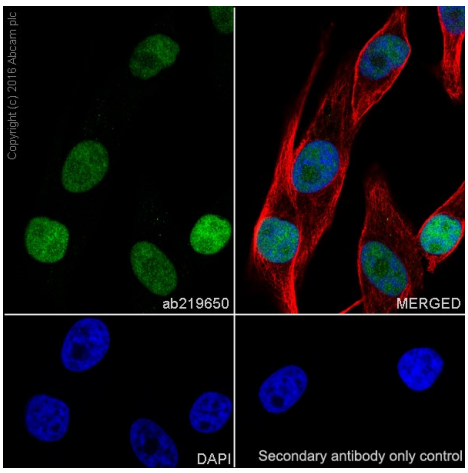
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed and 90% Methanol-permeabilised MeWo (Human malignant melanoma cell line) cells labeling PRAME with **ab219650** at 1/500 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).



Western blot - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using **ab219650**, the same antibody clone in a different buffer formulation. Different batches of **ab219650** were tested on MeWo (Human malignant melanoma fibroblast) whole cell lysate at 0.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 50 kDa.



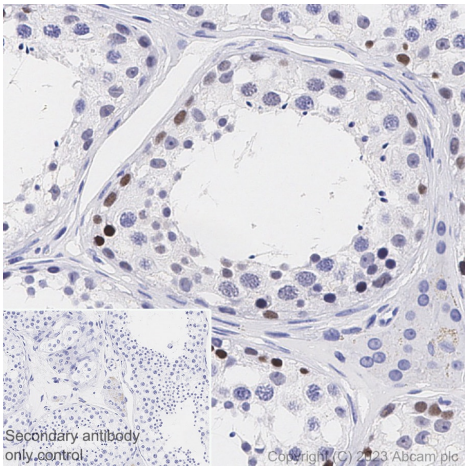
Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MeWo (Human malignant melanoma cell line) cells labeling PRAME with **ab219650** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing mostly nuclear staining on MeWo cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).



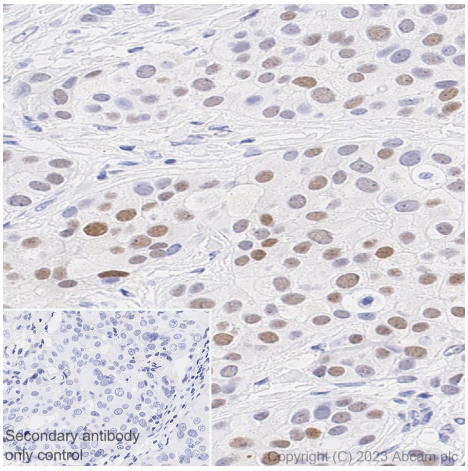
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Nuclear staining on human testis. The section was incubated with **ab219650** for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody.



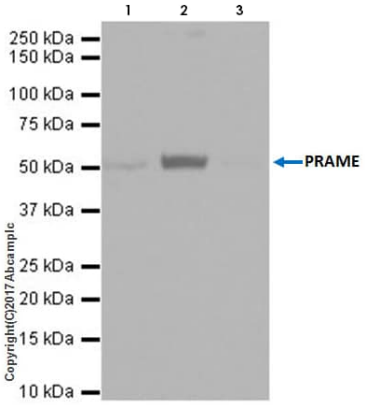
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Nuclear staining on human breast carcinoma. The section was incubated with **ab219650** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody.



Immunoprecipitation - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

PRAME was immunoprecipitated from 0.35 mg of MeWo (Human malignant melanoma cell line) whole cell lysate with **ab219650** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab219650** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: MeWo whole cell lysate 10 µg (Input).

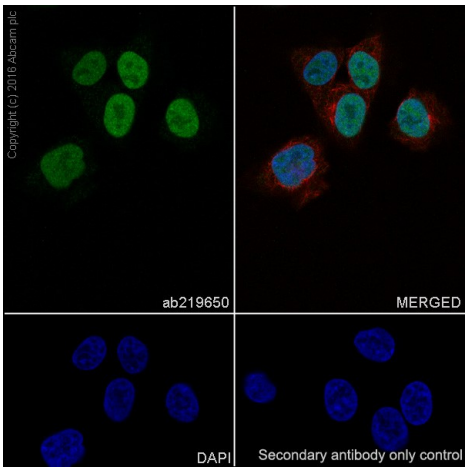
Lane 2: **ab219650** IP in MeWo whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab219650** in MeWo whole cell lysate.

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

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).



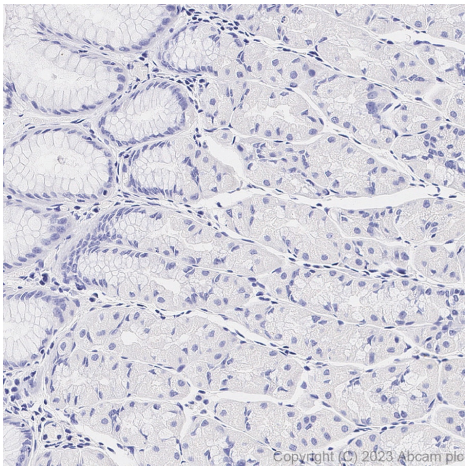
Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A-375 (Human malignant melanoma cell line) cells labeling PRAME with **ab219650** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing mostly nuclear staining on A-375 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

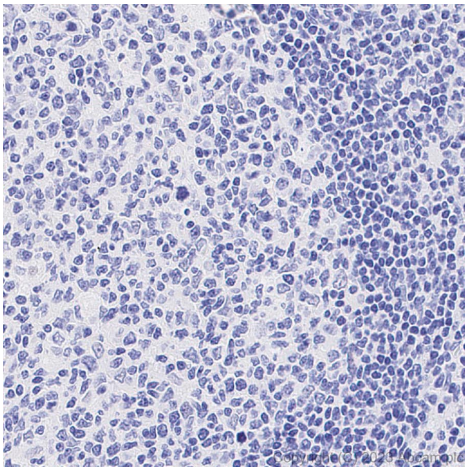


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Negative control: No staining on human stomach. The section was incubated with **ab219650** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

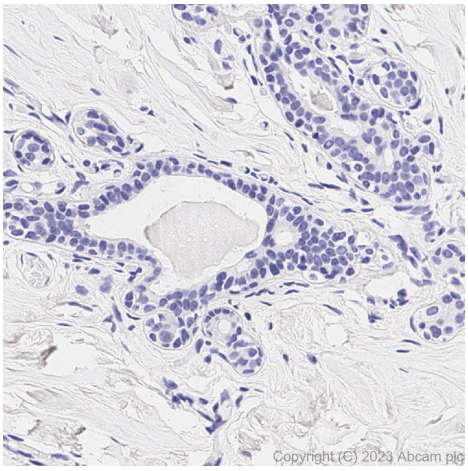


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Negative control: No staining on human tonsil. The section was incubated with **ab219650** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

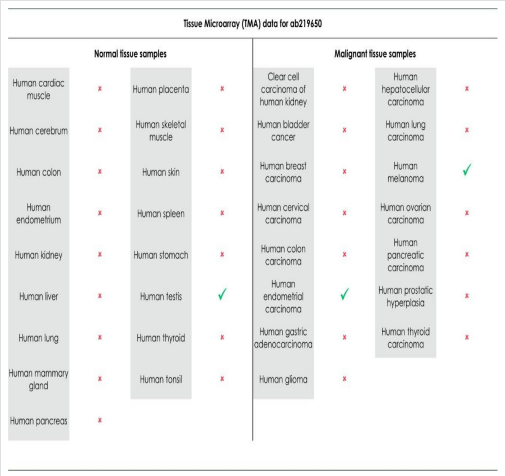


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab219650**).

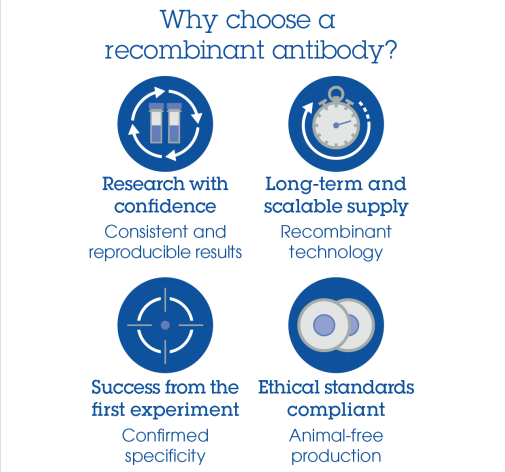
Immunohistochemical analysis of paraffin-embedded human breast tissue labeling PRAME with **ab219650** at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Negative control: No staining on human breast. The section was incubated with **ab219650** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

Tissue Microarrays stained for " Anti-PRAME antibody [EPR20330]" using " **ab219650**" 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 **ab93684** (Tris/EDTA buffer, pH 9.0). The sections were incubated with **ab219650** at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



Anti-PRAME antibody [EPR20330] - BSA and Azide free (ab232571)

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