abcam

Product datasheet

Anti-PRAME antibody [EPR20330] ab219650

Recombinant RabMAb

11 References 12 Images

Overview

Product name	Anti-PRAME antibody [EPR20330]
Description	Rabbit monoclonal [EPR20330] to PRAME
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:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
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 testis and melanoma tissues. ICC/IF: MeWo and A-375 cells. Flow Cyt (intra): MeWo cells. IP: MeWo whole cell lysate.
General notes	PRAME (PReferentially expressed Antigen in MElanoma) is a tumor-associated antigen and is a member of the family of cancer testis antigens (CTA). PRAME is expressed in malignant cells, including leukaemias, Hodgkin's lymphoma, breast cancer, and primary and metastatic melanomas. For more information, please refer to PMID: 27441500. PRAME has low or no expression in normal tissues except for in testis, ovary, placenta, adrenals and endometrium. For more information, please refer to PMID: 9047241. 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 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 (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20330
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab219650 in the following tested applications.

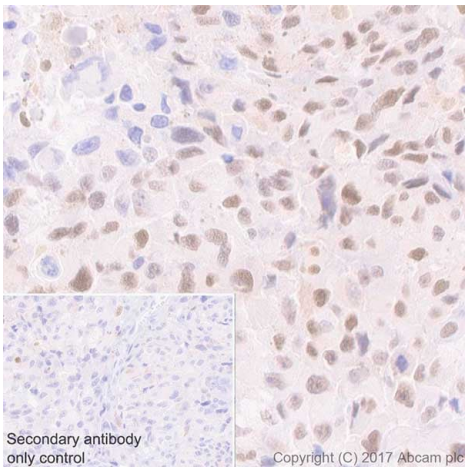
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 57 kDa (predicted molecular weight: 57 kDa).
IHC-P		1/16000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/30.
Flow Cyt (Intra)		1/500.

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



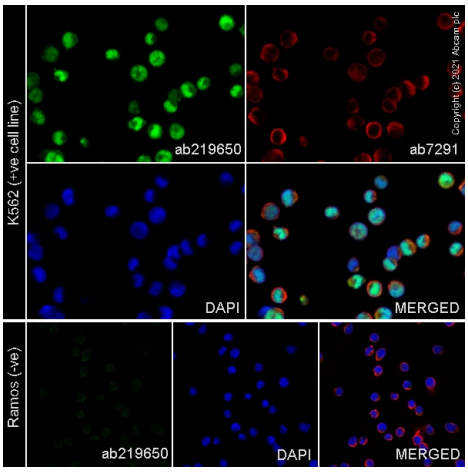
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling PRAME with ab219650 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining on human melanoma (PMID: 9047241).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

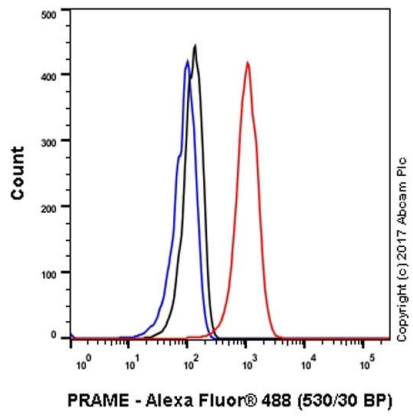


Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

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).

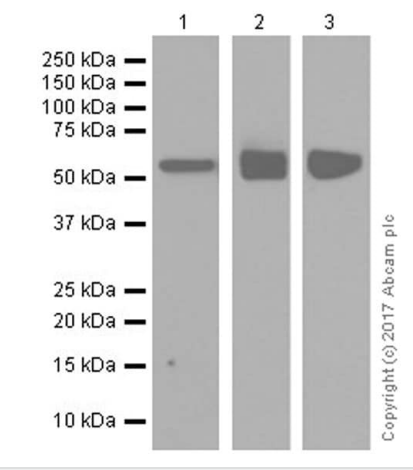
Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

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



Flow Cytometry (Intracellular) - Anti-PRAME antibody [EPR20330] (ab219650)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed 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.



Western blot - Anti-PRAME antibody [EPR20330] (ab219650)

All lanes : Anti-PRAME antibody [EPR20330] (ab219650) at 1/1000 dilution

Lane 1 : Human ovary cancer lysate
Lane 2 : A-375 (Human malignant melanoma cell line) whole cell lysate
Lane 3 : Human testis lysate

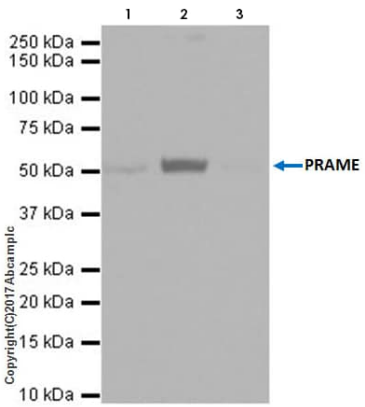
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Predicted band size: 57 kDa
Observed band size: 57 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2: 5 seconds; Lane 3: 1 minute.



Immunoprecipitation - Anti-PRAME antibody [EPR20330] (ab219650)

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.

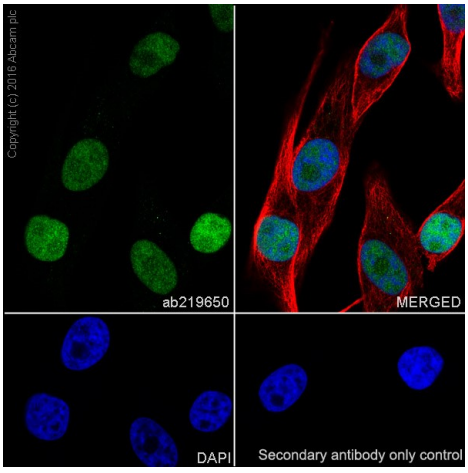
Lane 1: MeWo whole cell lysate 10 µg (Input).

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.



Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

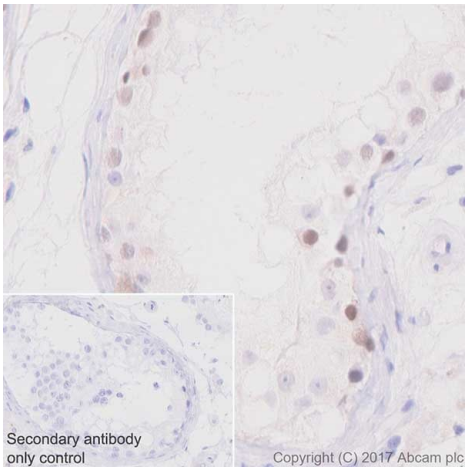
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MeWo (Human malignant melanoma cell line) cells labeling PRAME with ab219650 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal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

Western blot - Anti-PRAME antibody [EPR20330] (ab219650)

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.



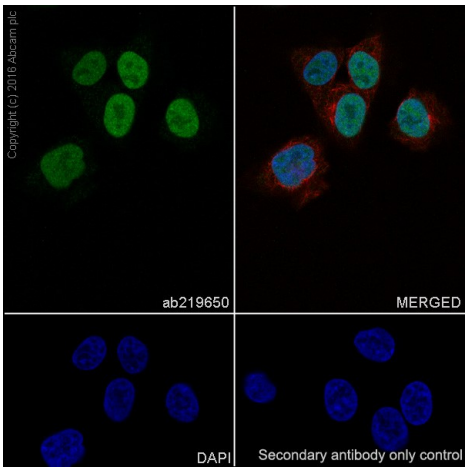
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling PRAME with ab219650 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining on human testis (PMID: 9047241).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

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.

Western blot - Anti-PRAME antibody [EPR20330] (ab219650)

Anti-PRAME antibody [EPR20330] (ab219650) at 1/1000 dilution + MeWo (Human malignant melanoma cell line) whole cell lysate at 20 µg

Secondary

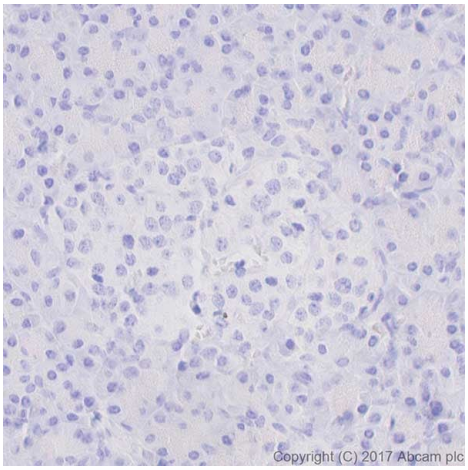
Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/4000 dilution

Predicted band size: 57 kDa

Observed band size: 57 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



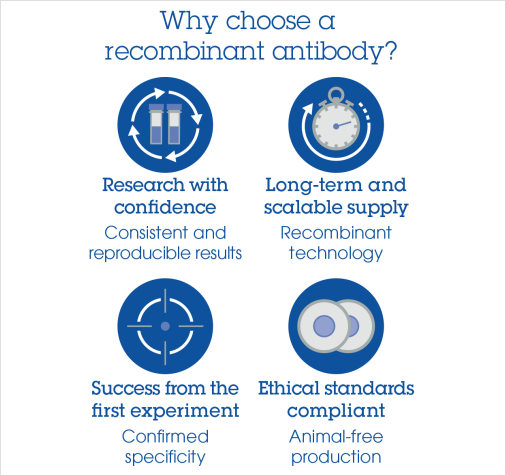
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling PRAME with ab219650 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on human pancreas (PMID: 9047241).

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-PRAME antibody [EPR20330] (ab219650)

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