abcam

ab219650

Anti-PRAME antibody [EPR20330]

Anti-PRAME antibody [EPR20330] (ab219650) is a rabbit monoclonal antibody detecting PRAME in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human** samples.

Enhanced IHC validation data - Download the data
- detailed IHC expression profiling in normal and clinically relevant cancer FFPE TMAs- melanoma, ovary and lung
- protocols for BOND RX™ and Ventana DISCOVERY ULTRA
Most cited clone to PRAME
Over 20 publications
Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20330
Purification technique	Affinity purification Protein A
Specificity	PRAME is expressed in malignant cells, including leukaemias, Hodgkin's lymphoma, breast cancer, and primary and metastatic melanomas.
Concentration	0.51 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/100 - 1/500

Notes	Recommend ab219650 incubation at +4°C overnight. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information PRAME

Function	Substrate-recognition component of a Cul2-RING (CRL2) E3 ubiquitin-protein ligase complex, which mediates ubiquitination of target proteins, leading to their degradation (PubMed:21822215, PubMed:26138980). The CRL2(PRAME) complex mediates ubiquitination and degradation of truncated MSRB1/SEPX1 selenoproteins produced by failed UGA/Sec decoding (PubMed:26138980). In the nucleus, the CRL2(PRAME) complex is recruited to epigenetically and transcriptionally active promoter regions bound by nuclear transcription factor Y (NFY) and probably plays a role in chromstin regulation (PubMed:21822215). 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 (PubMed:16179254).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-PRAME antibody [EPR20330] (ab219650) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, IP and WB.

Abcam's high quality manufacturing and validation processes ensure Anti-PRAME antibody [EPR20330] (ab219650) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-PRAME antibody [EPR20330] (ab219650) specifically detects PRAME (UniProt ID: P78395; Molecular weight:

58kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR20330 - ab232571.

Antibody clone EPR20330 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 555, Alexa Fluor® 647, Alexa Fluor® 488, Alexa Fluor® 594 (ab307626, ab307630, ab307769, ab312896).

This top cited antibody is highly specific for PRAME, a protein that is highly expressed in different types of cancers and is involved in cell proliferation and metastasis, as well as the outcomes of patients with cancer.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	PRAME or Preferentially Expressed Antigen in Melanoma is a cancer/testis antigen with a molecular mass of approximately 57 kDa. The PRAME protein is expressed in various tumors including melanoma and shows limited expression in normal adult tissues. Researchers use Monoclonal Antibody EPR20330 to detect PRAME due to its specificity and reliability in applications like PRAME immunohistochemistry (IHC).
Biological function summary	The PRAME protein influences cell proliferation and apoptosis by acting as a repressor of retinoic acid signaling. It is not part of a large complex but interacts with proteins involved in retinoic acid pathways. PRAME inhibits retinoic acid receptor signaling leading to disrupted cell differentiation and survival which is advantageous for cancer cells.
Pathways	Studies show the involvement of PRAME in important cellular processes. It plays a significant role in the retinoic acid pathway and interacts with retinoic acid receptors RAR and RXR. By inhibiting these receptors PRAME affects the transcriptional activity and cellular responses typically mediated by retinoic acid contributing to tumor growth and cancer cell survival.
Associated diseases and disorders	PRAME expression correlates with aggressive forms of melanoma and various other malignancies such as lung cancer. The presence of PRAME serves as a marker for poor prognosis and can indicate a more invasive cancer phenotype. In melanoma PRAME interacts with proteins related to the disease's progression further emphasizing its importance as a target for therapeutic strategies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

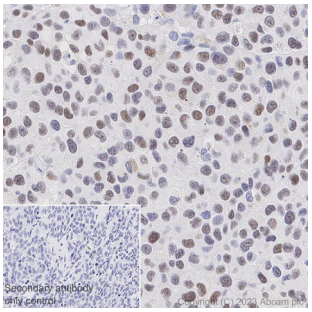
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

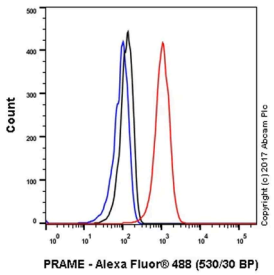
16 product images



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

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human melanoma tissue using rabbit Anti-PRAME antibody

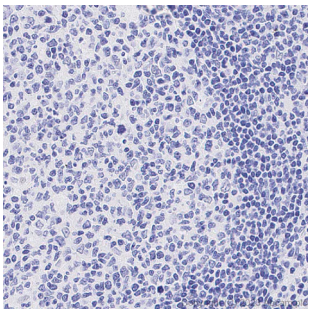
Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling PRAME with ab219650 at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before commencing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



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

PRAME Flow Cytometry (Intracellular) staining of MeWo (Human malignant melanoma cell line) cells using rabbit Anti-PRAME antibody

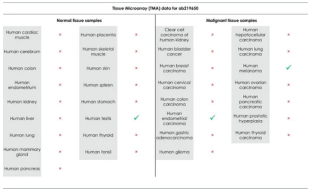
PRAME flow cytometry with PRAME antibody ab219650 of 4% paraformaldehyde-fixed and 90% Methanol-permeabilised MeWo (Human malignant melanoma cell line) cells labeling PRAME with ab219650 at 1/500 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabeled control (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.



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

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil using rabbit Anti-PRAME antibody

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.

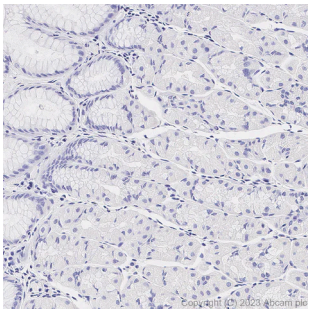


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

Tissue microarray (TMA) analysis using PRAME EPR20330 (ab219650).
Click here to view EPR20330 staining performance on human normal and cancer tissue microarray (TMA).

This table provides a detailed overview of positive PRAME staining (tick mark) and negative PRAME staining (cross mark) per sample type tested. PRAME is expressed in metastatic melanoma (PMID: 30045064). PRAME has low or no expression in normal tissues except in testis, ovary, placenta, adrenals and endometrium (PMID: 30045064).

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).

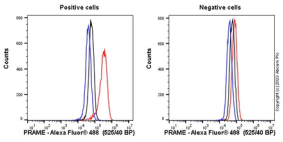


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human stomach using rabbit Anti-PRAME antibody

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.



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

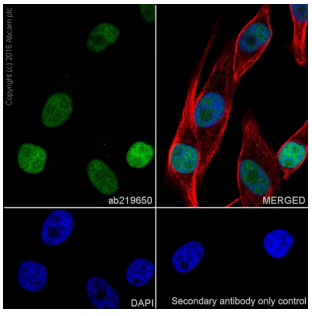
PRAME Flow Cytometry (Intracellular) staining using rabbit Anti-PRAME antibody

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 dilution)) 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.

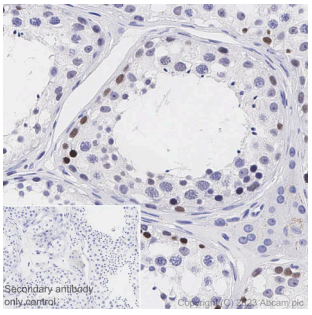


Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Immunocytochemistry/ Immunofluorescence staining of MeWo (Human malignant melanoma cell line) cells using rabbit Anti-PRAME antibody

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.

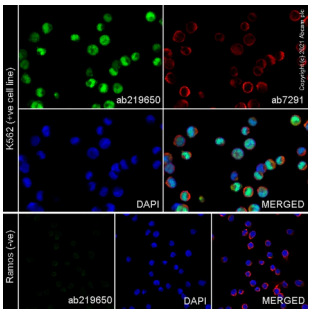


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human testis using rabbit Anti-PRAME antibody

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.

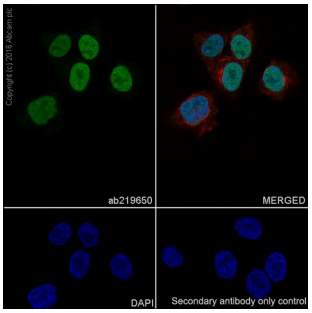


Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PRAME antibody

PRAME immunofluorescence with PRAME antibody ab219650 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.

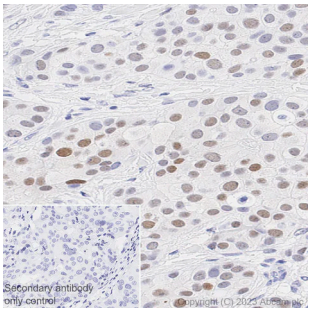


Immunocytochemistry/ Immunofluorescence - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Immunocytochemistry/ Immunofluorescence staining of A-375 (Human malignant melanoma cell line) cells using rabbit Anti-PRAME antibody

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).

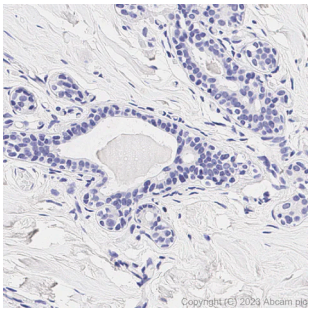
Secondary antibody only control: Used PBS instead of primary antibody secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



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

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human breast carcinoma using rabbit Anti-PRAME antibody

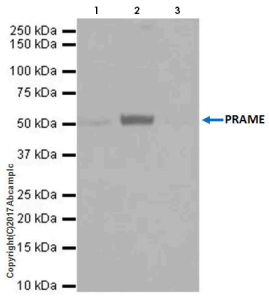
Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling PRAME with ab219650 at 1/500 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection). Sections were counter stained with Hematoxylin. Antigen retrieval was heat mediated with Tris/EDTA buffer pH 9.0 before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



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

PRAME Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human breast using rabbit Anti-PRAME antibody

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.



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.

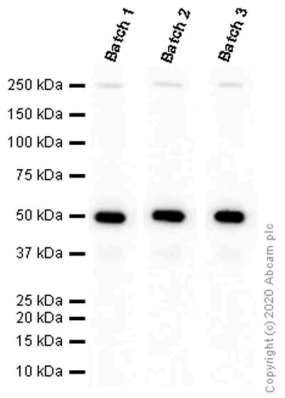
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab219650 in MeWo whole cell lysate.

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

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-PRAME antibody [EPR20330] (ab219650)

Predicted band size: 57 kDa



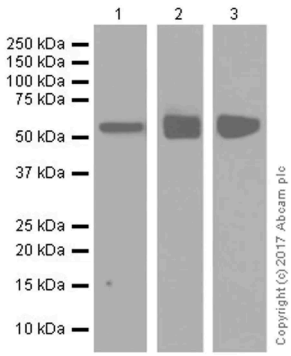
Western blot - Anti-PRAME antibody [EPR20330] (ab219650)

PRAME Western blot staining using rabbit Anti-PRAME antibody

PRAME western blot with PRAME antibody 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.

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

Predicted band size: 57 kDa



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

PRAME Western blot staining using rabbit Anti-PRAME antibody

PRAME western blot with PRAME antibody ab219650.
Blocking/Dilution buffer: 5% NFDM/TBST.

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

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

Lane 1:
Human ovary cancer lysate at 20 µg

Lane 2:
A-375 (Human malignant melanoma cell line) whole cell lysate at 20 µg

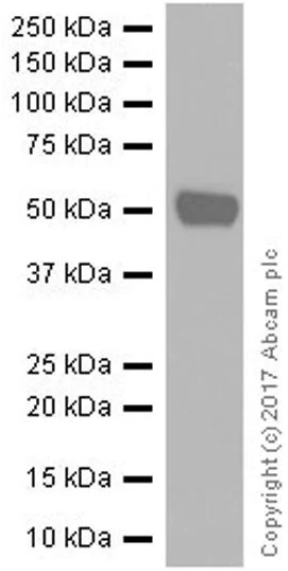
Lane 3:
Human testis lysate at 20 µg

Secondary

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

Predicted band size: 57 kDa

Observed band size: 57 kDa



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

PRAME western blot with PRAME antibody ab219650.
Blocking/Dilution buffer: 5% NFDM/TBST.

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

All lanes:
MeWo (Human malignant melanoma cell line) whole cell lysate at 20 µg

Secondary

All lanes:
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: 15s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.