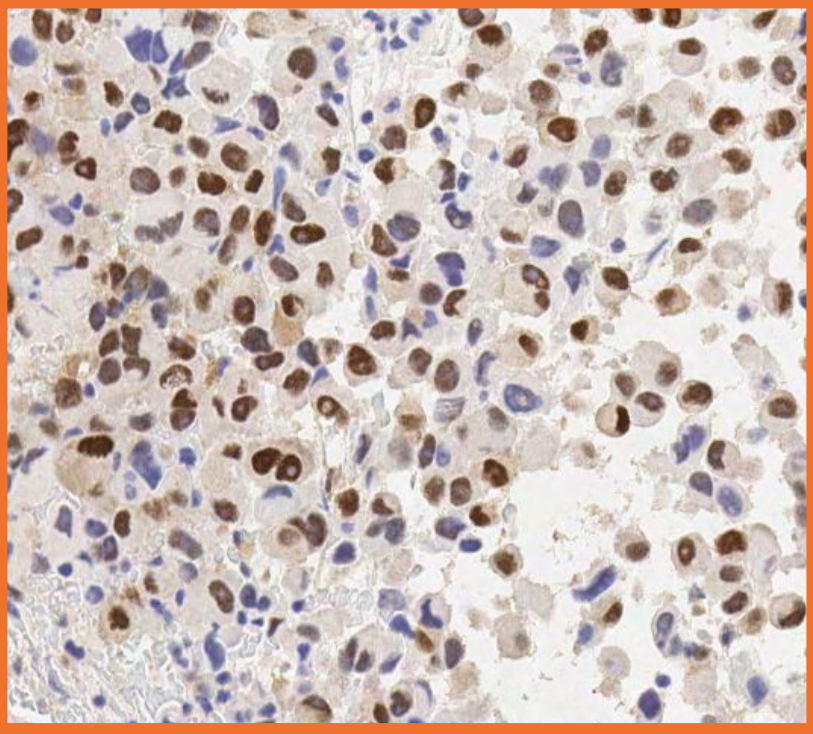
Enhanced validation data

Anti-PRAME recombinant antibody – ab219650



AB0329 2024/01 A

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Enhanced validation of Anti- PRAME recombinant antibody [EPR20330] – ab219650

Enhanced validation designed for your needs

We understand the challenge of finding the right antibody clone – highly specific and sensitive to your intended target – at early selection stages of your development program. To de-risk this clone selection process for you, we generated enhanced validation data for our best recombinant antibody clones to some of the most promising targets.

Our enhanced validation gives you an extra level of confidence in an antibody clone

— Provides additional data on the specificity and sensitivity of our recombinant antibodies in immunohistochemistry (IHC) and other relevant techniques

— Carried out in a custom manner, specific both to the target and the relevant research & clinical settings

— Builds upon our high-quality standard validation

Our framework for enhanced validation

— Our enhanced validation focuses on generating detailed IHC expression profiles for promising immuno-oncology targets in selected formalin-fixed paraffin-embedded (FFPE) human normal tissues and cancer tissue microarrays (TMAs).

— In this study, we demonstrate the sensitivity and specificity of Anti- PRAME recombinant antibody [EPR20330] – ab219650 in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) (results in figures 1-5).

— An assay was also developed using the DISCOVERY ULTRA system (results in figures 6 and 7).

Target overview

HGNC symbol
PRAME

Approved name
PRAME nuclear receptor transcriptional regulator

Previous symbols:
Preferentially expressed antigen in melanoma
4930534P07Rik antibody
Cancer/testis antigen 130 antibody

Chromosomal location:
22q11.22

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.

Cellular localization

Cell membrane. Expressed at the cell surface. A soluble form has also been detected.

Target information above from: UniProt accession P78395
The UniProt Consortium
The Universal Protein Resource (UniProt) in 2010
Nucleic Acids Res. 38:D142-D148 (2010)

Materials and methods

Human FFPE tissues					
Tissue microarray (TMA)	**Cores**	**Cases**	**Normal/ Benign cases**	**Cancer cases**	**Source (#catalog number)**
Multi normal	34	34	34	0	Pantomics (#MEL1021)
Melanoma cancer	102	102	5	97	Pantomics (#MEL1021)
Ovary cancer	102	102	5	97	Pantomics (#OVC1021)
Lung cancer	102	102	5	97	Pantomics (#LUC1021)

Table 1. List of human FFPE TMAs used in the enhanced validation. All tissues were sourced from Abcam-approved tissue suppliers.

Prestaining protocol		
Step	**Reagents**	**Pre-programmed protocol**
Dewax	Bond™ dewax solution (AR922), alcohol, BOND wash solution (AR9590)	Dewax
Antigen retrival	Bond™ epitope retrieval ER1 solution (AR9961)	HIER with ER1 (pH 5.9–6.1), 20 minutes, 100°C

Table 2a. IHC prestaining protocol on BOND™ RX Research Stainer (Leica®).

Staining protocol			
Step	**Reagents**	**Number of washes**	**Time (minutes)**
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Recombinant anti-PRAME rabbit monoclonal [EPR20330] antibody – ab219650 diluted in Bond™ primary antibody diluent (AR9352) to final concentration of 5µg/mL	-	15
Wash	Bond™ wash solution	3x	0
Secondary detection	Bond™ polymer refine detection (DS9800)	-	8
Wash	Bond™ wash solution	2x	4
	Deionized water	1x	0
Visualization	Mixed DAB refine (DS9800)	1x	0
	Mixed DAB refine (DS9800)	-	10
Wash	Deionized water	3x	0
Counterstain	Hematoxylin (DS9800)	-	5
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

Table 2b. IHC staining protocol on BOND™ RX Research Stainer (Leica®). The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature.

Step	Reagents	Method
Deparaffinization	EZ Prep	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	64 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Recombinant anti-PRAME rabbit monoclonal [EPR20330] antibody – ab219650 diluted in Bond™ primary antibody diluent (AR9352) to final concentration of 2.9µg/mL	32 minutes, 36 °C
Counterstain	Hematoxylin II	8 min
Post counterstain	Bluing reagent	4 min

Table 3. IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument. Staining was performed using standard conditions with OptiView DAB IHC Detection Kit.

Percentage of IHC-positive tumor cells (A)	Intensity of IHC reaction (B)
0 = 0%	0 = no reaction
1 = <30%	1 = weak
2 = 30-60%	2 = moderate
3 = >60%	3 = strong
Final score = A x B (range 0-9)	

Table 4. IHC scoring method. A semi-quantitative IHC scoring method was used to determine the expression of PRAME in tumor cells (Table 5). This method assessed the extent of IHC staining using the proportion and intensity of stained tumor cells in TMA cores[1]. Results were analyzed on GraphPad Prism using box or scatter plots to show the distribution of scores. Incomplete cores or those with less than 50% tumor cells were excluded from the analysis.

PRAME expression in normal human tissues (BOND™ RX)

PRAME expression was detected in the seminiferous ducts and cell membrane of the Leydig cells of the testis and skin sebocytes[2], as expected. Expression was absent in the ovary, lung and skin epidermis.

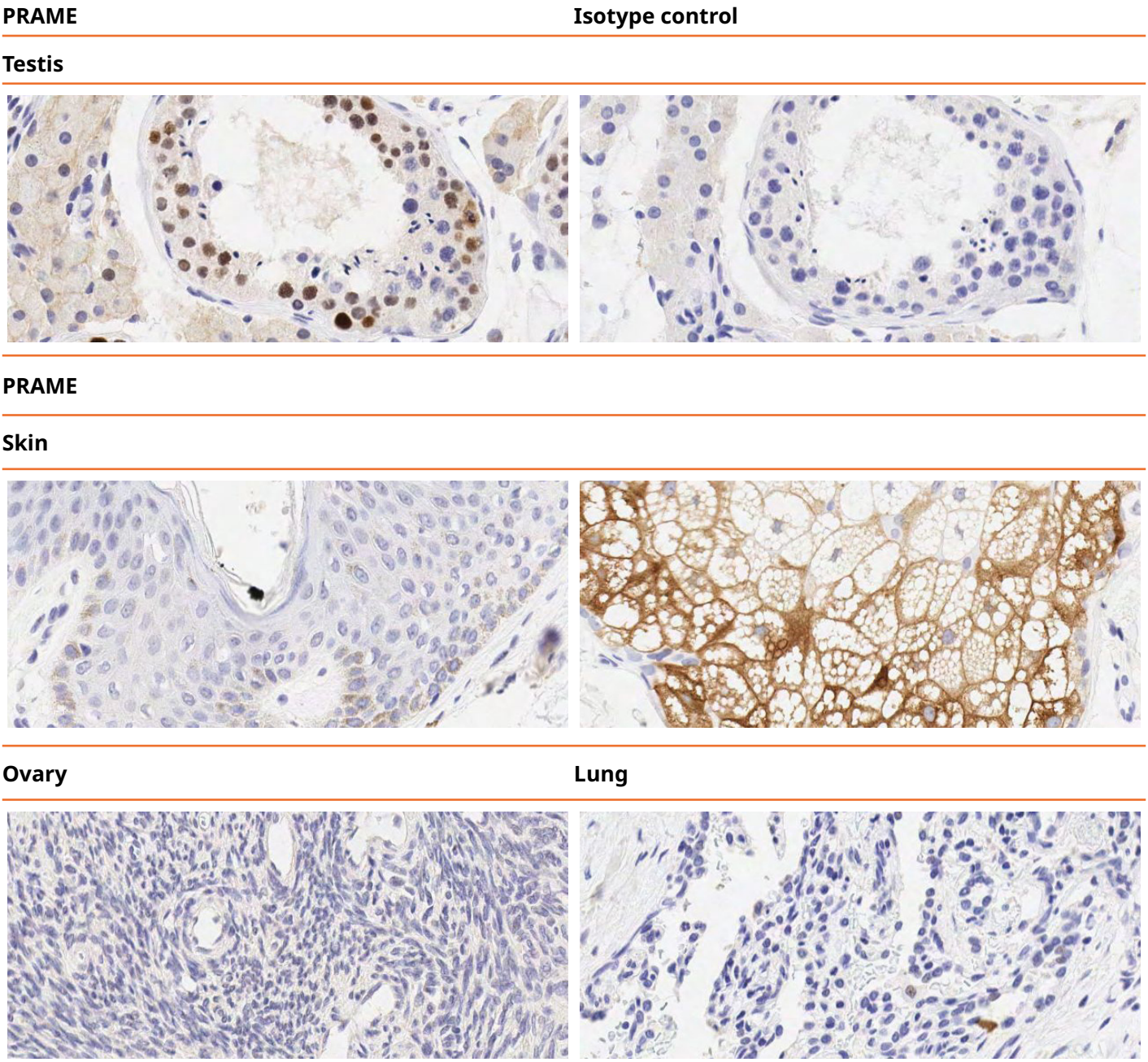


Figure 1. PRAME expression in human normal tissue. IHC staining of normal human testis, skin, ovary and lung tissue using anti-PRAME (ab219650) or rabbit IgG–isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

PRAME expression in cancer (BOND™ RX)

PRAME expression varied in the analyzed cancer tissue micorarrays (TMAs), with melanoma showing the highest IHC score and lung cancer the lowest. The staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD).

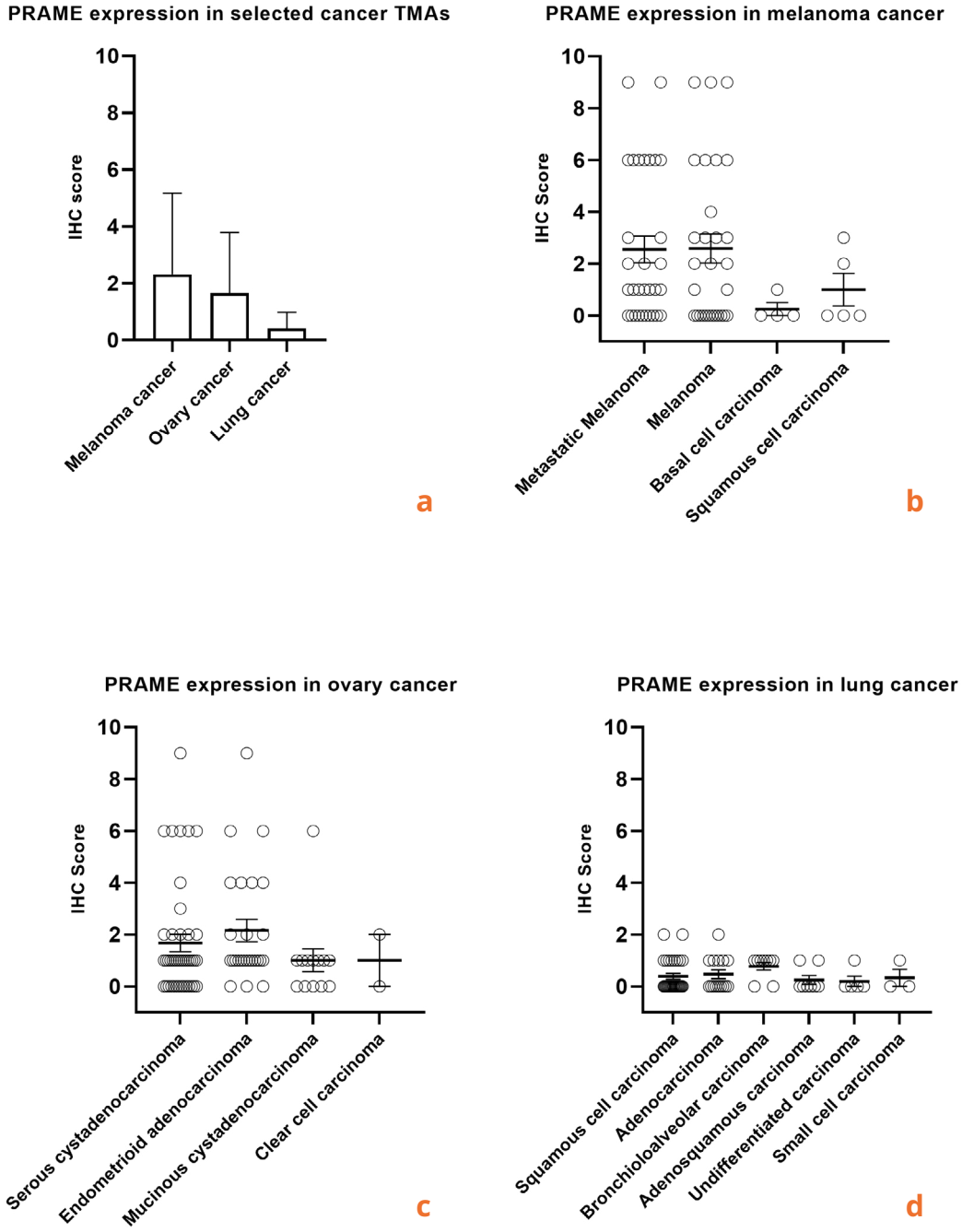
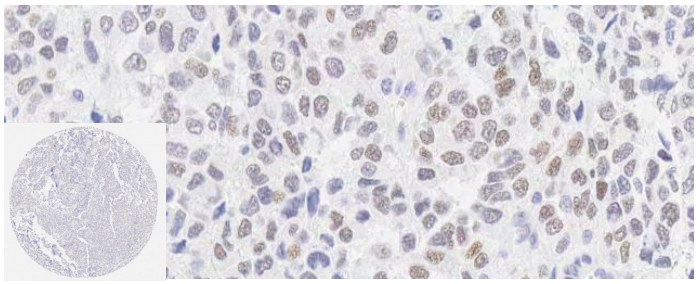


Figure 2. PRAME protein expression in a selection of cancer TMAs. (a) The box plot (with SD) summarizes results from a semi-quantitative analysis of PRAME expression in TMA cores. (b) PRAME IHC score in 31 TMA cores of metastatic melanoma, 29 TMA cores of melanoma, 5 TMA cores of basal cell carcinoma and 4 TMA cores of squamous cell carcinoma. (c) PRAME IHC score in 43 TMA cores of serous cystadenocarcinoma, 26 TMA cores of endometrioid adenocarcinoma, 13 TMA cores of mucinous cystadenocarcinoma and 2 TMA cores of clear cell carcinoma. (d) PRAME IHC score in 31 TMA cores of squamous cell carcinoma, 15 TMA cores of adenocarcinoma, 9 TMA cores of bronchioloalveolar carcinoma, 7 TMA cores adenosquamous carcinoma, 5 TMA cores undifferentiated carcinoma, 3 TMA cores small cell carcinoma.

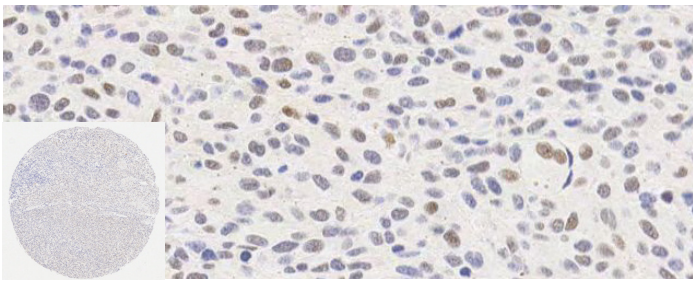
PRAME expression in melanoma cancer TMA (BOND™ RX)

Below are the representative images of individual melanoma cancer cases showing weak to strong PRAME expression.

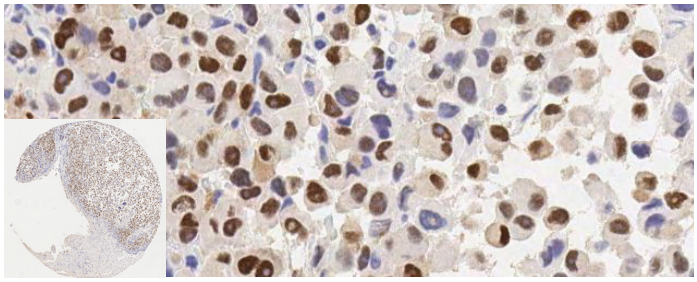
Melanoma
(weak PRAME expression)



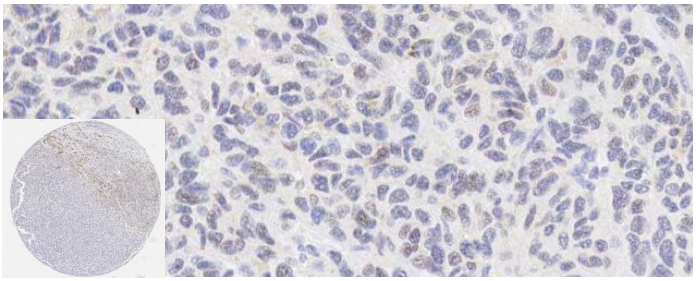
Melanoma
(moderate PRAME expression)



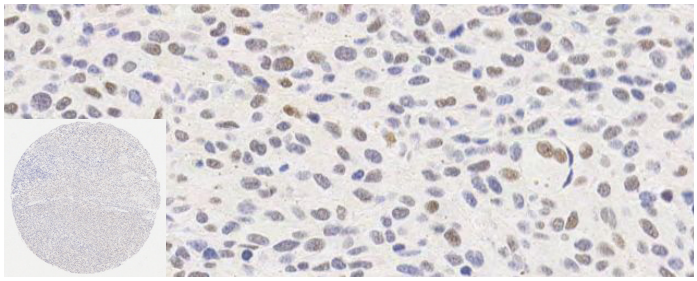
Melanoma
(strong PRAME expression)



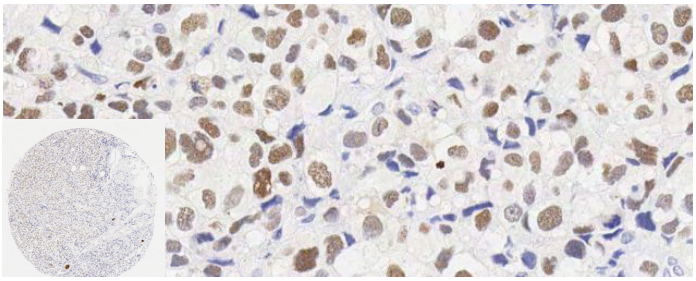
Metastatic melanoma
(weak PRAME expression)



Metastatic melanoma
(moderate PRAME expression)



Metastatic melanoma
(strong PRAME expression)



Squamous cell carcinoma
(weak PRAME expression)

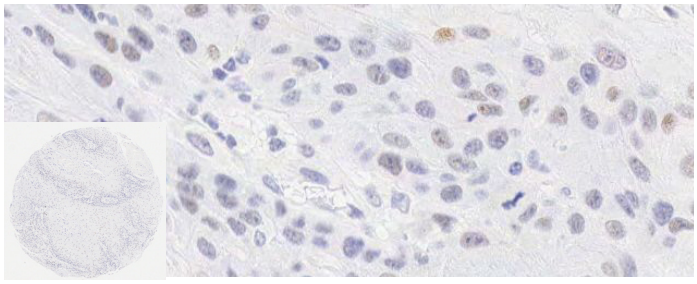
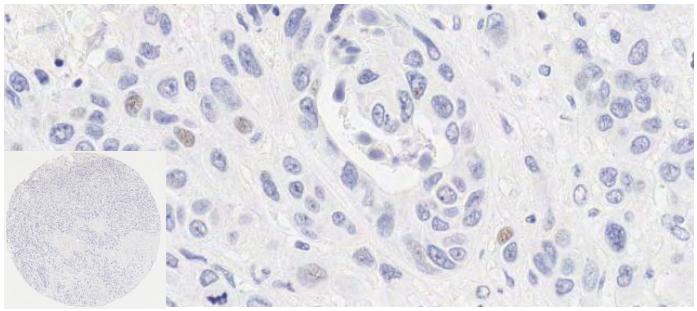


Figure 3. PRAME expression in melanoma. IHC images show weak, moderate or strong PRAME staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

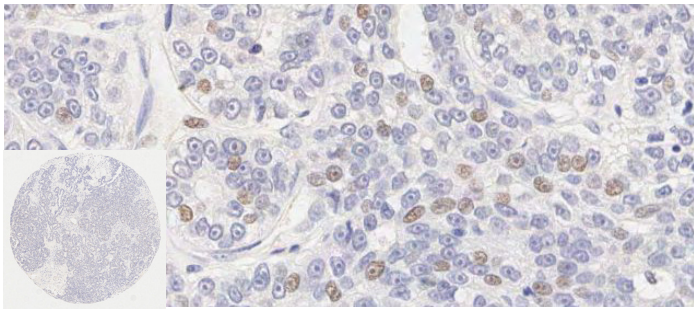
PRAME expression in ovarian cancer TMA (BOND™ RX)

Below are the representative images of weak to strong PRAME expression in individual cases of endometroid adenocarcinoma and serous cystadenocarcinoma and weak PRAME expression in one case of mucinous cytadenocarcinoma (Figure 4).

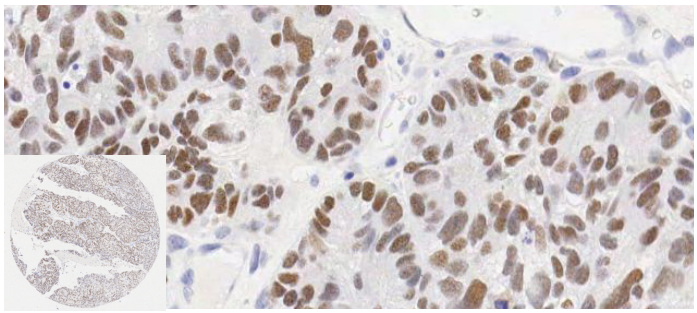
Endometrioid adenocarcinoma
(weak PRAME expression)



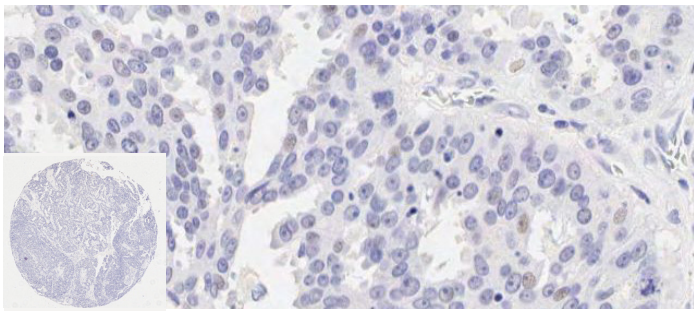
Endometrioid adenocarcinoma
(moderate PRAME expression)



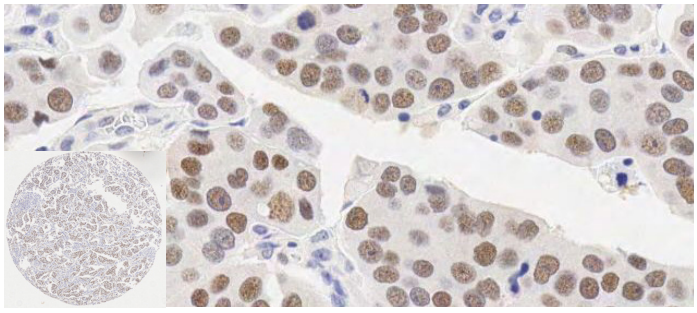
Endometrioid adenocarcinoma
(strong PRAME expression)



Serous cystadenocarcinoma
(weak PRAME expression)



Serous cystadenocarcinoma
(strong PRAME expression)



Mucinous cystadenocarcinoma
(weak PRAME expression)

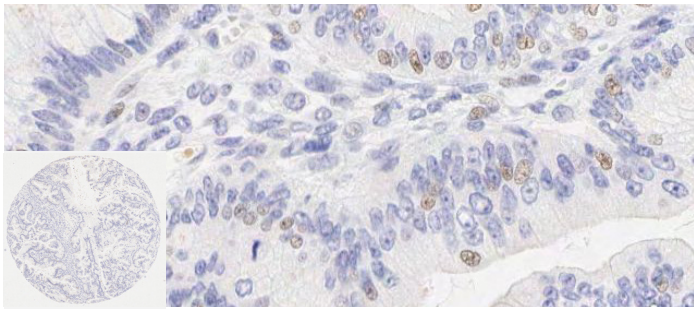
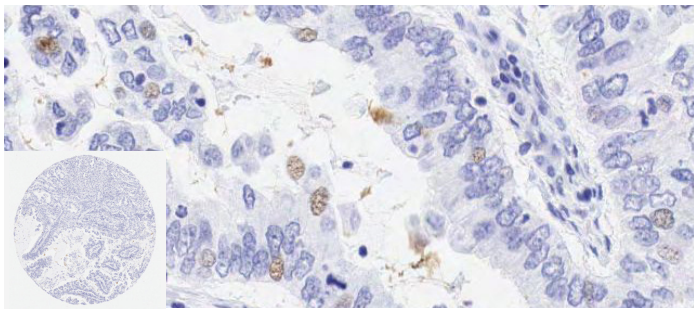


Figure 4. PRAME expression in ovarian cancer. IHC images show weak, moderate or strong PRAME staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

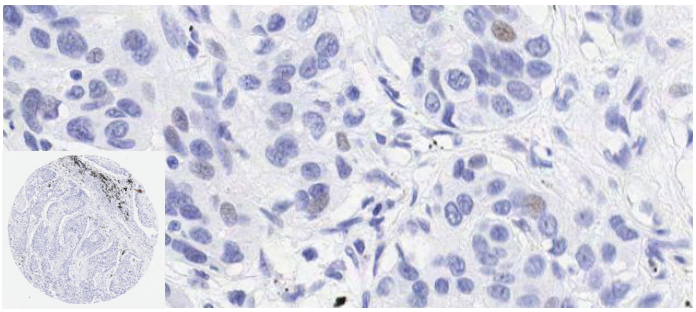
PRAME expression in lung cancer TMA (BOND™ RX)

Below are the representative images of individual cases of lung cancer showing weak to moderate PRAME expression (Figure 5).

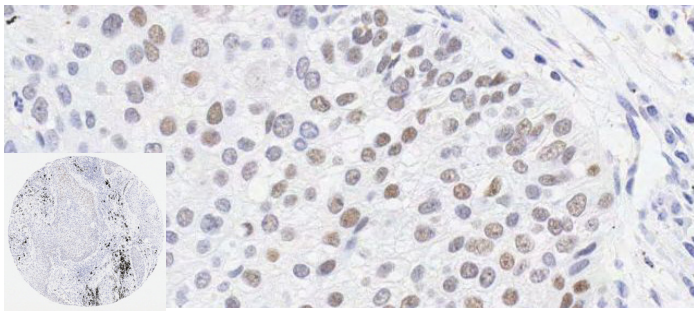
Adenocarcinoma
(weak PRAME expression)



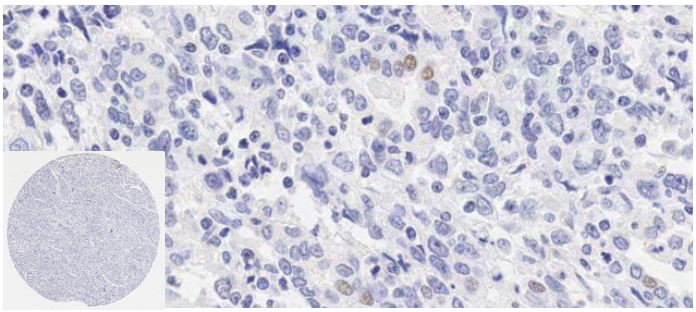
Squamous cell carcinoma
(weak PRAME expression)



Squamous cell carcinoma
(moderate PRAME expression)



Adenosquamous carcinoma
(weak PRAME expression)



Bronchioloalveolar carcinoma
(weak PRAME expression)

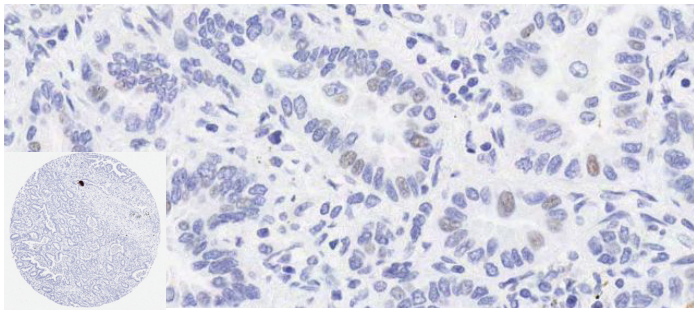


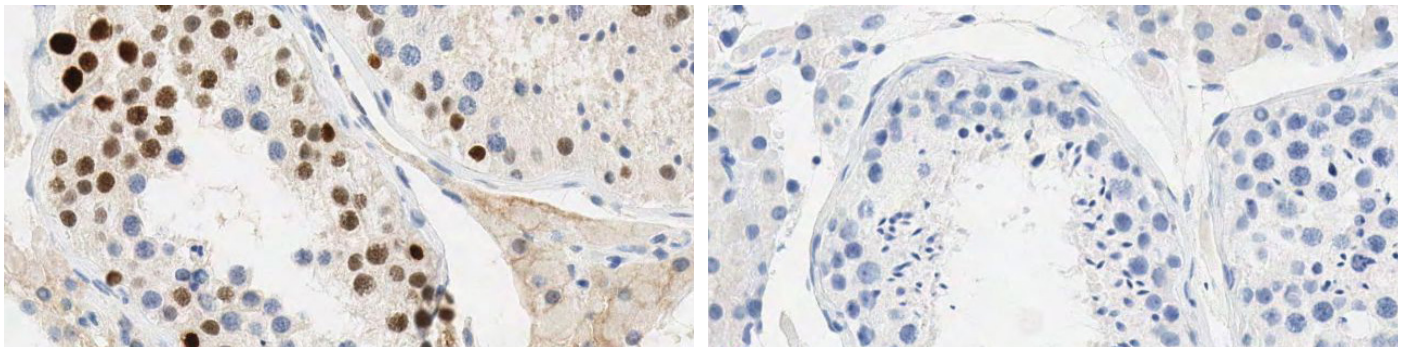
Figure 5. PRAME expression in lung. IHC images show weak, moderate or strong PRAME staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

PRAME expression in normal human tissues (DISCOVERY ULTRA)

PRAME expression was detected in the seminiferous ducts and cell membrane of the Leydig cells of the testis and skin sebocytes[2], as expected. No expression was detected in the ovary, lung and skin epidermis.

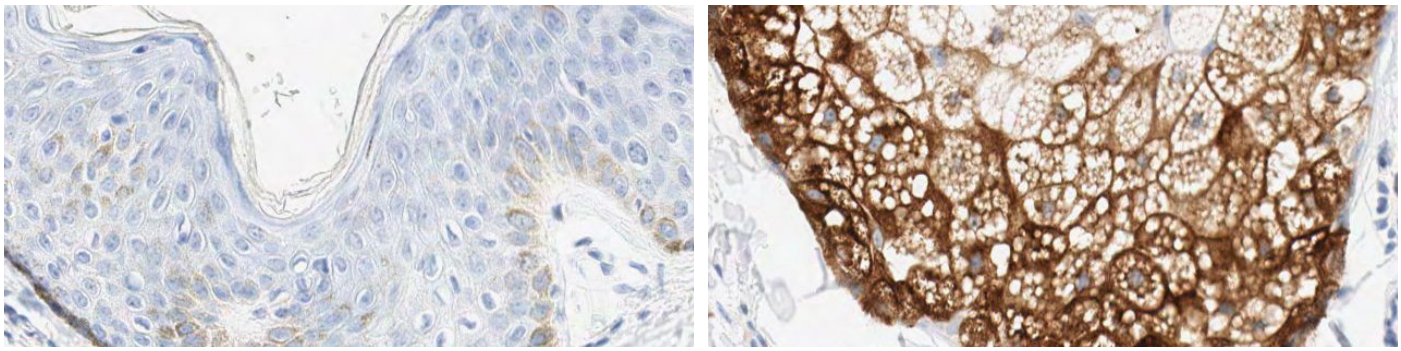
PRAME | **No primary**

Testis



PRAME

Skin



Ovary | **Lung**

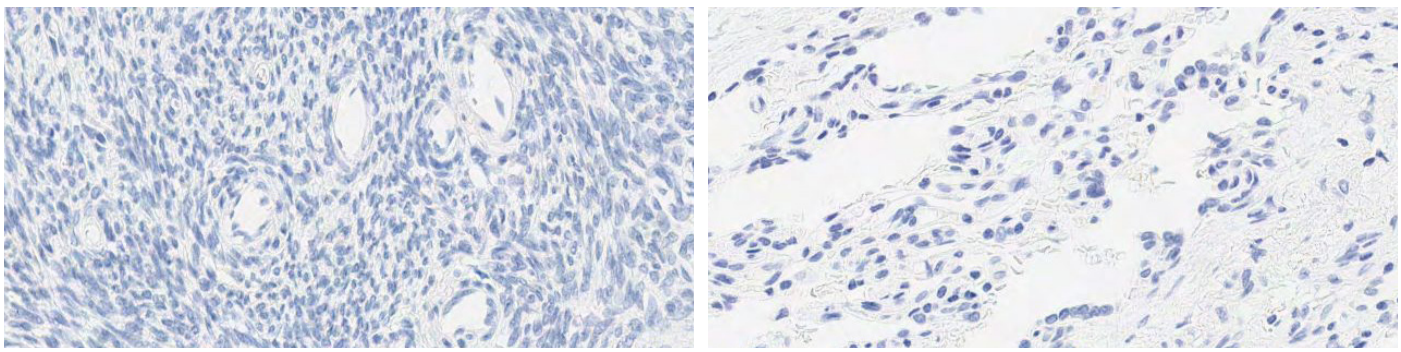
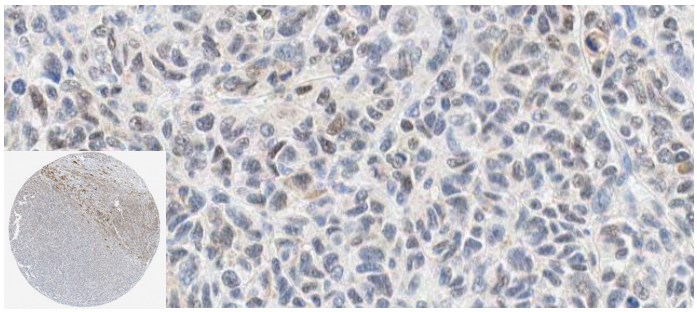


Figure 6. PRAME expression in human normal tissue. IHC staining of normal human testis, skin, ovary and lung tissue using anti-PRAME (ab219650) or no primary antibody using the DISCOVERY ULTRA platform. Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

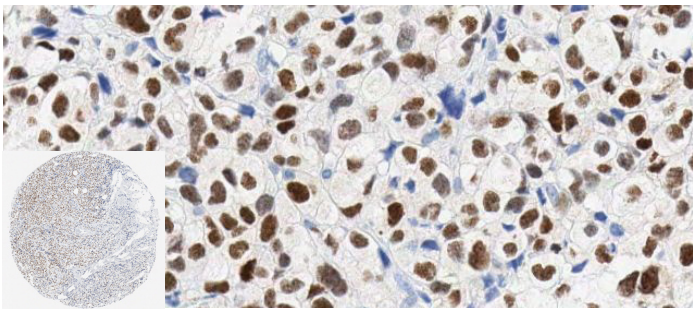
PRAME expression in cancer TMA (DISCOVERY ULTRA)

Below are the representative images of cancer cases stained with the DISCOVERY ULTRA, showing weak to strong PRAME expression.

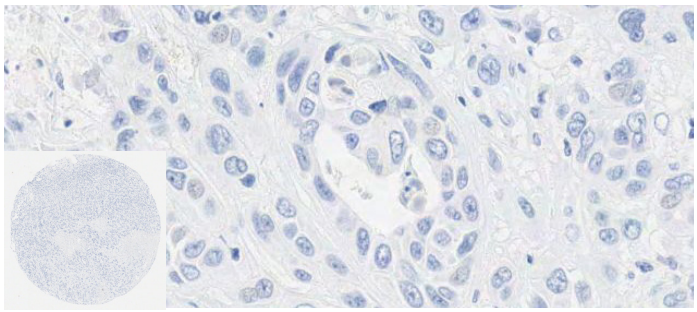
Metastatic melanoma
(weak PRAME expression)



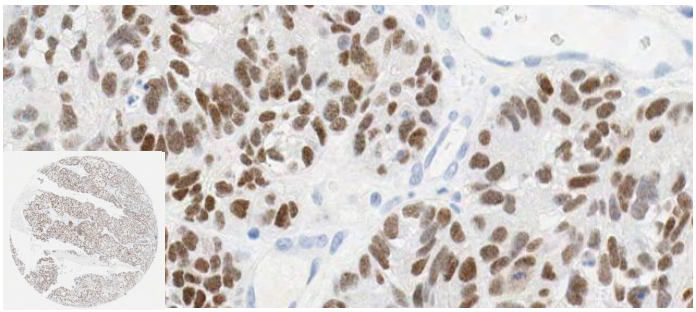
Metastatic melanoma
(strong PRAME expression)



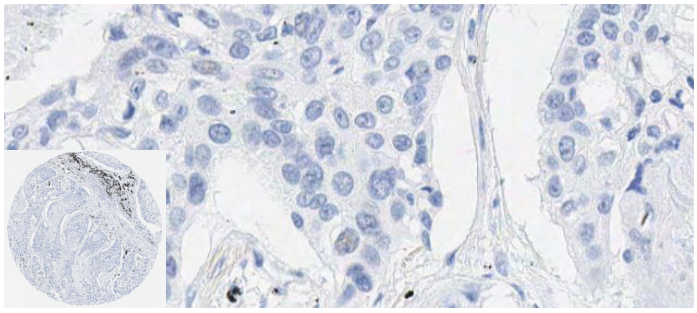
Endometrioid adenocarcinoma
(weak PRAME expression)



Endometrioid adenocarcinoma
(strong PRAME expression)



Lung squamous cell carcinoma
(weak PRAME expression)



Lung squamous cell carcinoma
(moderate PRAME expression)

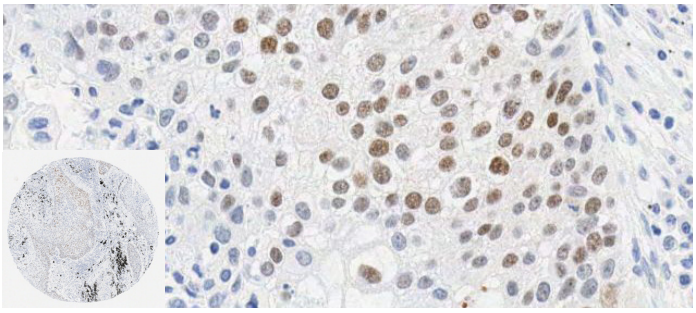


Figure 7. PRAME expression in lung. IHC images show weak, moderate or strong PRAME staining intensity in brown stained on the DISCOVERY ULTRA. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

References

1. Fedchenko, N., & Reifenrath, J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. *Diagnostic pathology*, **9**, 221 (2014).

2. Lezcano, C., Jungbluth, A. A., Nehal, K. S., Hollmann, T. J. & Busam, K. J. PRAME expression in melanocytic tumors. *American Journal of Surgical Pathology* **42**, 1456–1465 (2018).

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