

PRAME [EPR20330]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3252-060321

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3252 A, B	0.1, 0.5 mL	1:100	Van Gogh Yellow
Predilute	API 3252 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3252 T60	60 tests	Ready-to-use	N/A
Q Series-- For Leica BOND-III	ALI 3252 G7	7.0 mL	Ready-to-use	N/A
UltraLine -- For BenchMark	AVI 3252 G, G25	6.0, 25mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

PRAME [EPR20330] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of PRAME protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

PRAME (preferentially expressed antigen in melanoma) is located on chromosome 22q11.22 and encodes a 509 amino acid protein. PRAME is an autosomal cancer-testis antigen (CTA) gene. PRAME is expressed in melanoma, various nonmelanocytic malignant neoplasms, including non-small cell lung cancer, breast carcinoma, renal cell carcinoma, ovarian carcinoma, leukemia, synovial sarcoma, and myxoid liposarcoma. Normal healthy tissues are not known to express PRAME except for testis, ovary, placenta, adrenals, and endometrium. (1,2)

PRAME is one of the most widely studied CTAs and has been associated with the outcome and risk of metastasis. PRAME is currently being investigated as a novel immunotherapy target and diagnostic marker with ongoing clinical trials that include Hodgkin's disease, leukemia, multiple myeloma, breast cancer, pancreatic cancer, brain cancer. (3,4)

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit monoclonal

Species Reactivity: Human, others not tested

Clone: EPR20330

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: PRAME

Cellular Localization: Nucleus and cell membrane

Positive Tissue Control: Melanoma, normal testis

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker data sheet for specific instructions.

Protocol Recommendations (intelliPATH FLX and manual use)

Cont'd:

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI3252 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: PRAME Rb

Protocol Template (Description): Rb HRP Template 1 – OR – Rb AP Template 1

Dewaxing (DS Buffer Option): DS Buffer

Antigen Retrieval (AR Option): AR1, high pH; 103°C

Block Option: Buffer

Reagent Name, Time, Temp.: PRAME Rb, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI3252 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 64 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 32 minutes, 36°C

Protocol Recommendations (O Series – For Leica BOND-III):

ALI3252 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F

Detection: Bond Polymer Refine

HIER: 20 min with ER2

Peroxide Block: 5 min

Marker (Primary Antibody): 15 min

Post Primary: 8 min

Polymer: 8 min

Mixed DAB Refine: 10 min

Hematoxylin: 5 min

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Protocol Recommendations (Q Series – For Leica BOND-III)

Cont'd:

-Red Chromogen Staining Option:

Protocol Name: IHC Protocol J

Detection: Bond Polymer Refine Red

HIER: 20 min with ER2

Marker (Primary Antibody): 15 min

Post Primary AP: 20 min

Polymer AP: 30 min

Mixed Red Refine: 10 min + 5 min

Hematoxylin: 5 min

Performance Characteristics:

Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (5)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Zhang W, *et al.* PRAME expression and promoter hypermethylation in epithelial ovarian cancer. *Oncotarget*, 2016 Jul; 7(29):45352-69.
2. Lezcano C, *et al.* PRAME expression in melanocytic tumors. *Am J Surg Pathol*. 2018 Nov; 42(11):1456-65.
3. Salmaninejad A, *et al.* Cancer/testis antigens: expression, regulation, tumor invasion and use in immunotherapy cancers. *Immunol Invest*. 2016 Oct; 45(7):619-40.
4. Al-Khadairi G, Decock J. Cancer testis antigens and immunotherapy: where do we stand in the targeting of PRAME? *Cancers*. 2019 Jul; 11(7):984.
5. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."

References Cont'd:

6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Melanoma	31	40
Ovary Cancer	7	45
Breast Cancer	8	26
Colon Cancer	0	36
Lung Cancer	17	51
Prostate Cancer	11	40
Adrenocortical carcinoma	0	1
Bladder Cancer	0	2
Meningioma	0	2
Astrocytoma	0	1
Squamous Cell carcinoma (esophagus)	0	2
Adenocarcinoma (stomach)	0	2
Adenocarcinoma (small intestine)	0	1
Adenocarcinoma (colon & rectum)	0	6
Kidney Cancer	0	2
Liver Cancer	0	4
Lymphoma	3	6
Squamous Cell carcinoma (head & neck, oral cavity, tongue)	0	1
Nasopharyngeal carcinoma	1	1
Adenocarcinoma (pancreas)	0	1
Adenocarcinoma (prostate)	0	2
Adenoid cystic carcinoma (head and neck, salivary gland)	0	1
Squamous Cell carcinoma (skin)	0	1
Seminoma	0	2
Thyroid Cancer	0	2
Cervical Cancer	0	2
Endometrium Cancer	1	2

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Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	3	3
Cerebellum	0	3
Adrenal	2	3
Ovary	1	11
Pancreas	0	3
Parathyroid	0	3
Pituitary	0	2
Testis	7	8
Thyroid	0	4
Breast	3	3
Spleen	0	3
Tonsil	0	3
Thymus	0	2
Bone Marrow	1	2
Lung	0	3
Heart	0	3
Esophagus	3	4
Stomach	0	4
Small Intestine	0	4
Colon	2	13
Liver	0	4
Salivary Gland	0	3
Kidney	0	4
Prostate	2	11
Uterus	0	3
Cervix	0	2
Skeletal Muscle	0	3
Skin	2	10
Peripheral Nerve	0	2
Lining Cells	0	2
Bladder	0	1
Head, neck and salivary gland	0	1
Lymph node	0	1