p16 + PRAME

Prediluted Multiplex Antibody Reagent
901-3256DS-050222

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	API 3256DS AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3256DS T60	60 tests	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3256DS G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
p16 + PRAME is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of p16 INKa and PRAME proteins 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:
p16 INK4a is a tumor suppressor protein involved in the pathogenesis of a variety of malignancies and is a 16.5 kDa protein expressed in the nucleoplasm of proliferating cells, functioning as an inhibitor of CDK4. Recent analyses of the p16INK4a gene revealed homozygous deletions, nonsense, missense, or frameshift mutations in several human cancers. p16 INK4a gene has been reported among melanomas, gliomas, esophageal, pancreatic, lung, and urinary bladder carcinomas (1,2).
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 not only expressed in melanoma, but also 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 (3,4).
An optimized antibody cocktail for p16 INK4a + PRAME may aid in the clear distinction between nodal nevi from nodal metastatic melanoma. p16 has been shown to exhibit lower expression in metastatic melanoma, while PRAME has been shown to have greater expression in metastatic melanoma; p16 expression is retained in nevi (5,6).

Principle of Procedure:
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:
p16 + PRAME is provided as a prediluted antibody cocktail of anti-p16 and anti-PRAME antibodies in buffer with carrier protein and preservative.

Antibody	anti-p16	anti-PRAME
Clone	BC42	EPR20330
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG
Epitope/ Antigen	p16 INK4a	PRAME
Cellular Localization	Nuclear and cytoplasmic	Nucleus and cell membrane
Staining	Brown (DAB)	Red

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Melanoma

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker product data sheet for specific instructions.
Protein Block: Incubate for 10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Double Stain Detection: Incubate for 30 minutes at RT using MACH 2 Double Stain 2.
Chromogen (1): Incubate for 5 minutes at RT with Betazoid DAB.
Chromogen (2): Incubate for 7 minutes at RT with Warp Red. Rinse in deionized water.
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 2 Double Stain 2 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):
OPAI3256DS 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: p16 + PRAME
Protocol Template (Description): Multiplex 2 Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR1, high pH; 101°C
Block Option: Buffer
Reagent Name, Time, Temp.: p16 + PRAME, 30 min., 25°C

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3256DS 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: Parallel DS Protocol K
Detection: ChromoPlex 1 Dual IHC
HIER: 20 min with ER2
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Polymer mHRP: 8 min
Polymer rAP: 20 min
Mixed DAB Refine: 10 min
Mixed Red Refine 2: 10 + 5 min
Hematoxylin: 5 min

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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 (NaN_3) 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) (7)
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. (8)
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. LaPak KM, Burd CE. The molecular balancing act of p16(INK4a) in cancer and aging. Mol Cancer Res. 2014 Feb; 12(2):167-83.
2. Ikenberg H, *et al.* Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: results of the PALMS study. J Natl Cancer Inst. 2013; 105:1550–7.
3. Zhang W, *et al.* PRAME expression and promoter hypermethylation in epithelial ovarian cancer. Oncotarget. 2016 Jul; 7(29):45352-69.
4. Lezcano C, *et al.* PRAME expression in melanocytic tumors. Am J Surg Pathol. 2018 Nov; 42(11):1456–65.
5. See S, Finkelman B, Yeldandi A. Diagnostic Utility of p16 and PRAME in Differentiating Nodal Nevi from Nodal Metastatic Melanoma. Nature 2020; 33(2):499.
6. Serra S, Chetty R. *p16.* Journal of Clinical Pathology. 2018; 71:853-8.
7. 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."
8. 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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