p16 INK4a [BC42]

Concentrated and Prediluted Monoclonal Antibody
901-3231-060123

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3231 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	API 3231 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3231 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 3231 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3231 G, G25	6.0, 25 mL	Ready-to-use	N/A
Q Series – For Leica BOND-III	ALI 3231 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
p16 INK4a [BC42] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of p16 INK4a 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:
p16 INK4a is a tumor suppressor protein involved in the pathogenesis of a variety of malignancies. It is a specific inhibitor of cdk4/cdk6. Recent analyses of the p16INK4a gene revealed homozygous deletions, nonsense, missense, or frameshift mutations in several human cancers (1). Although the frequency of p16 INK4a abnormalities is higher in tumor-derived cell lines than in unselected primary tumors, significant subsets of clinical cases with aberrant p16 INK4a gene have been reported among melanomas, gliomas, esophageal, pancreatic, lung, and urinary bladder carcinomas (2). p16 immunoreactivity in paraffin-embedded tissues has also been shown to be an independent predictor in minimally invasive urothelial bladder cancer; a prognostic factor in non-small cell lung carcinoma; and has been shown to predict a positive response to chemoradiotherapy in Stage IV head and neck squamous cell carcinoma (3-6).

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-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human, others not tested
Clone: BC42
Isotype: IgG1/kappa
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: p16 INK4a
Cellular Localization: Nuclear and cytoplasmic
Positive Tissue Control: Normal tonsil, cervical cancer, head and neck cancer and colon cancer

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTM3231 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: Incubate for 10 minutes with Val Mouse Secondary.
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 10 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 60 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 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):
OPAI3231 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
Protocol Template (Description): Ms HRP 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, 59 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):
AVI3231 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 48 minutes

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

IVD CE

Rev: 062117

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Protocol Recommendations (Ventana BenchMark ULTRA) Cont'd:
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 12 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3231 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

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 (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. Mahajan A. Practical issues in the application of p16 immunohistochemistry in diagnostic pathology. Hum Pathol. 2016 May; 51:64-74.
3. Tong J, *et al.* Expression of p16 in non-small cell lung cancer and its prognostic significance: A meta-analysis of published literatures. Lung Cancer. 2011 Nov; 74(2):155-63.
4. Chen YJ, *et al.* High p16 expression predicts a positive response to chemoradiotherapy in stage IVa/b head and neck squamous cell carcinoma. Asian Pac J Cancer Prev. 2011; 12(3):649-55.
5. Snow AN, Laudadio J. Human papillomavirus detection in head and neck squamous cell carcinomas. Adv Anat Pathol. 2010 Nov; 17(6):394-403.
6. Buza N, *et al.* Inverse p16 and p63 expression in small cell carcinoma and high-grade urothelial cell carcinoma of the urinary bladder. Int J Surg Pathol. 2010 Apr; 18 (2):94-102.
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.

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

Tissue	Positive Cases	Total Cases
Cervical intraepithelial neoplasia	16	24
Cervical adenocarcinoma	13	22
Cervix squamous cell carcinoma	16	16
Head and neck cancer	4	12
Bladder Cancer	28	39
Breast Cancer	26	29
Colon Cancer	28	35
Lung Cancer	25	48
Endometrium cancer	42	48
Ovarian Cancer	10	12
Prostate Cancer	10	12
Renal Cancer	21	30

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60 Berry Drive
Pacheco, CA 94553
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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	3	3
Adrenal	3	3
Ovary	2	3
Pancreas	3	3
Parathyroid	1	3
Pituitary	3	3
Testis	0	3
Thyroid	0	3
Breast	3	3
Spleen	3	3
Tonsil	3	3
Thymus	3	3
Bone Marrow	3	3
Lung	0	3
Heart	0	3
Esophagus	2	3
Stomach	3	3
Small Intestine	2	3
Colon	1	3
Liver	1	3
Salivary Gland	3	3
Kidney	2	3
Prostate	3	3
Uterus	2	3
Cervix	1	3
Skeletal Muscle	0	2
Skin	3	3
Peripheral Nerve	1	3
Linging Cells	*	3

* + in lung; - in muscle & fat

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