p16 + Ki-67

Prediluted Multiplex Antibody Reagent
901-3246DS-050620

BIOCARE MEDICAL

Catalog Number:	**API 3246DS AA, H**	**VLTMR 3246 G20**
Description:	6.0, 25 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
p16 + Ki-67 is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of p16 and Ki-67 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. p16 INK4a is a 16.5 kDa protein expressed in the nucleoplasm of proliferating cells, functioning as an inhibitor of CDK4. Recent analyses of the p16 INK4a gene revealed homozygous deletions, nonsense, missense, or frameshift mutations in several human cancers (1).
The Ki-67 nuclear antigen is associated with cell proliferation. It is found throughout the cell cycle in the G1, S, G2, and M phases; but not the (G0) phase. It is commonly used to grade proliferation rates of tumors. An optimized antibody cocktail for p16 INK4a and Ki-67 may aid in the identification of cells co-expressing markers for both tumor suppression and cell proliferation, an indicator of cell-cycle deregulation (2). Co-expression of p16 and Ki-67 has been observed in cervical intraepithelial neoplasia (CIN) lesions associated with high risk HPV infection, presenting potential utility in the classification of CIN lesions (3).

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 + Ki-67 is provided as a prediluted antibody cocktail of anti-p16 and anti-Ki-67 antibodies in buffer with carrier protein and preservative.

Antibody	anti-p16	anti-Ki-67
Clone	BC42	SP6
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG
Epitope/ Antigen	p16 INK4a	Ki-67
Cellular Localization	Nuclear and cytoplasmic	Nuclear
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: Cervical cancer

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTMR3246 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-Hi pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block: Incubate for 10 minutes with Val Background Block.
Primary Antibody: Incubate for 45 minutes.
Double Stain Detection: Incubate for 30 minutes using Val Plex 2.
Chromogen (1): Incubate for 5 minutes with Val DAB.
Chromogen (2): Incubate for 15 minutes with Val Fast Red.
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 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 Notes:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 Double Stain 2. Use TBS for washing steps.

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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Rev: 062117

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

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) (4)
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. (5)
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. Rossi P, *et al*. A population of 1136 HPV DNA-HR positive women: expression of p16(INK4a)/Ki67 Dual-Stain Cytology and cytological diagnosis. Histological correlations and cytological follow up. Pathologica. 2015;107:185–91.
4. 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."
5. 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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

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