Ki-67

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-325-050218

BIOCARE MEDICAL

Catalog Number:	**CRM 325 A, B, C**	**PRM 325 AA, H**	**OAI 325 T60**
Description:	0.1, 0.5, 1.0 ml, concentrated	6.0, 25 ml, prediluted	60 tests, prediluted
Dilution:	1:50	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
Ki-67 [SP6] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of Ki-67 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:
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 (GO) phase. It is used to grade proliferation rates of tumors. The high affinity and/or binding capacity of rabbit antibodies provide superior staining results and less chance for technical false negatives.

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, an enzyme labeled polymer is added to bind to the primary antibody. This detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit monoclonal
Species Reactivity: Human, cat, cow, dog, mouse, pig, rat and sheep
Clone: SP6
Isotype: IgG
Total Protein Concentration: ~10 mg/ml. Lot specific Ig concentration is not available.
Epitope/Antigen: Ki-67
Cellular Localization: Nuclear
Positive Tissue Control: Tonsil or breast 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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH and manual use):
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare's Diva, Reveal or Rodent Decloaker. Refer to the respective retrieval product data sheet for specific instructions.
Protein Block (**Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.
Primary Antibody: Incubate for 30-60 minutes at RT.
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Protocol Recommendations (intelliPATH and manual use) Cont'd:
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Protocol Recommendations (ONCORE Automated Slide Staining System):
OAI325 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:
Protocol Name: Ki67 Rb
Protocol Template (Description): Rb HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR1, high pH; 103°C
Reagent Name, Time, Temp.: Ki67 Rb, 30 min., 25°C

Technical Notes:
1. This antibody, for intelliPATH and manual use, has been standardized with Biocare's MACH 2 detection system.
2. For mouse and rat tissue, Rodent Block M & R is recommended. If background staining is observed, XM or XR Factor may be added to detection. For details, see product data sheet.
3. For mouse and rat tissue, Rabbit-on-Rodent Detection is recommended. For dog and cat tissue, Rabbit-on-Canine detection is recommended. For detection protocol please see detection data sheet.
4. 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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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 an 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

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Precautions Cont'd:
volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
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 in contact with sensitive areas, wash with copious amounts of water. (7)
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. Jansen R, *et al.* MIB-1 labelling index is an independent prognostic marker in primary breast cancer. Br J Cancer. 1998 Aug; 78 (4):460-5.
2. Goodson WH, *et al.* The functional relationship between in vivo bromodeoxyuridine labeling index and Ki-67 proliferation index in human breast cancer. Breast Cancer Res Treat. 1998 May; 49 (2):155-64.
3. Rossi S, *et al.* A comparative study between a novel category of immunoreagents and the corresponding mouse monoclonal antibodies. Am J Clin Pathol. 2005; 124(2):295-302.
4. Pena LL, *et al.* Immunohistochemical detection of Ki-67 and PCNA in canine mammary tumors: relationship to clinical and pathologic variables. J Vet Diag Invest. 1998 Jul; 10 (3):237-46.
5. Gibbons D, *et al.* Comparison of topoisomerase II alpha and MIB-1 expression in uterine cervical squamous lesions. Mod Pathol. 1997 May; 10 (5):409-13.
6. 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."
7. 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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