

Ki-67

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-325-060223

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M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CRM 325 A, B, C	0.1, 0.5, 1.0 mL	1:50	Van Gogh Yellow
Predilute	PRM 325 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE	OAI 325 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 325 T60	60 tests	Ready-to-use	N/A
VALENT	VLTR 325 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 325 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 325 G7	7.0 mL	Ready-to-use	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 (G0) 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, 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, cat, cow, dog, mouse, pig, rat and sheep

Clone: SP6

Isotype: IgG

Protein Concentration: 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 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 (VALENT® Automated Slide Staining Platform):

VLTR325 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:

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

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 (Optional): Incubate for 10-20 minutes with Val Background Block.

Primary Antibody: Incubate for 30 minutes.

Secondary: N/A

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 20 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 Peroxidized 1.

Pretreatment: Perform heat retrieval using 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 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 Warp Red.

Counterstain:

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

Technical Notes:

1. This antibody, for intelliPATH FLX and manual use, has been standardized with 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.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI325 is intended for use with the ONCORE. Refer to the User Manual for specific instructions on its use. Protocol parameters in Protocol Editor should be programmed as follows:

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Protocol Recommendations (ONCORE Automated Slide Staining System) Cont'd:

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

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

OPA1325 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: Ki-67 Rb

Protocol Template (Description): Rb HRP Template 1

Dewaxing (DS Buffer Option): DS2-50

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

Block Option: Buffer

Reagent Name, Time, Temp.: Ki-67 Rb, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI325 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 32 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 32 minutes, 36°C

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

ALI325 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: 30 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

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) (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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