ROS1 [EPMGHR2]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3240-060123

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3240 A, B	0.1, 0.5 mL	1:100	Renoir Red
Predilute	API 3240 AA	6.0 mL	Ready-to-use	N/A
VALENT	VLTR 3240 G20	20 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3240 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
ROS1 [EPMGHR2] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of ROS1 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 *ROS1* gene encodes ROS proto-oncogene 1, a receptor tyrosine kinase. The ROS1 protein is an enzyme with a mass of 263.9 kDa. *ROS1* gene rearrangements have been implicated in multiple cancer indications including non-small cell lung cancer (NSCLC), gastric cancer, glioblastoma multiforme, ovarian cancer, and colorectal cancer (1). ROS1 protein fusion expression leads to oncogenic transformation, most likely through constitutive activation of the tyrosine kinase domain (2). *ROS1* gene rearrangements occur in 1-2% of NSCLC, and clinical trial data indicates that tumors positive for ROS1 protein fusions are sensitive to kinase inhibitors such as crizotinib, and entrectinib (3,4). For detection of positive ROS1 fusions, IHC testing has been shown 100% sensitivity and 92-94% specificity in recent studies, proving its efficacy as a screening tool for ROS1-targeted therapies (5,6).

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-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
Clone: EPMGHR2
Isotype: IgG
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: Synthetic peptide within Human ROS1
Cellular Localization: Cytoplasmic
Positive Tissue Control: Lung 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):
VLTR3240 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).
Enzyme: Incubate for 10 minutes with Val Zyme Pronase (1:25 mix)
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 60 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 Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product 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: 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 Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3240 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 ER1
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 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. Gainor JF, Shaw, AT. Novel Targets in Non-Small Cell Lung Cancer: ROS1 and RET Fusions. Oncologist. 2013;18(7):865-75.
2. Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res. 2013 Aug 1;19(15):4040-5.
3. Bubendorf L, *et al*. Testing for ROS1 in non-small cell lung cancer: a review with recommendations. Virchows Arch. 2016 Nov;469(5):489-503.
4. Facchinetti F, Friboulet L. Profile of entrectinib and its potential in the treatment of ROS1-positive NSCLC: evidence to date. Lung Cancer (Auckl). 2019 Sep 9;10:87-94.
5. Sholl LM, *et al*. ROS1 immunohistochemistry for detection of ROS1-rearranged lung adenocarcinomas. Am J Surg Pathol. 2013 Sep;37(9):1441-9.
6. Shan L, *et al*. Detection of ROS1 gene rearrangement in lung adenocarcinoma: comparison of IHC, FISH and real-time RT-PCR. PLoS One. 2015 Mar 5;10(3):e0120422.
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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