

PD-1 (RM)

Prediluted Rabbit Monoclonal Antibody
901-3162-053023

BIOCARE
M E D I C A L

Catalog Number:	API 3162 AA	VLTR 3162 G20
Description:	6.0 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

PD-1 (RM) [EP239] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of PD-1 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:

Programmed death 1 (PD-1) is a cell surface co-receptor in the CD28/CTLA-4 T-cell family and functions as a down regulator of the immune system through a dual mechanism of inhibition (1). PD-1 is expressed on the cell surface of activated T- and B-cells. Anti-tumor immunity may be controlled by the PD-1/PD-L1 signaling pathway. PD-L1, one of the ligands associated with PD-1, provides immunity for tumor cells by inducing apoptosis of activated T cells or by inhibiting cytotoxic T cells (1,2). Therapies that target the PD-1 receptor have shown unprecedented results with high levels of clinical response in patients with various cancer types (3). The presence of PD-1 positive tumor-infiltrating lymphocytes (TIL) has been associated with poor prognosis in human breast cancers and may be useful in antibody therapy targeting the PD-1/PD-L1 signaling pathway (1). Treatments targeting PD-1 and its ligand, PD-L1, have also shown encouraging results in non-small-cell lung cancer, renal cell carcinoma and melanoma (4-6). This antibody can also be used in multiplex stains with CD8, FOXP3, CK8/18 and MART-1 mouse monoclonal antibodies.

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; others not tested

Clone: EP239

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: A synthetic peptide corresponding to residues of human PD-1 protein

Cellular Localization: Cytoplasmic/cell membrane

Positive Tissue Control: Colon cancer and melanoma

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.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTR3162 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 (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 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 30 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:

1. This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 Rabbit and MACH 2 Double Stain 1 detection systems. Use TBS buffer for washing steps.

2. If this reagent is used in combination with other primary antibodies, the antibody incubation time may need to be extended up to 60 minutes, depending upon the particular protocol of the individual investigator.

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

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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 into 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. Muenst S, *et al.* The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. *Breast Cancer Res Treat.* 2013 Jun;139(3):667-76.
2. Kim JW, Eder JP. Prospects for Targeting PD-1 and PD-L1 in Various Tumor Types. *Oncology.* (Williston Park). 2014 Nov;28(11 Suppl 3).
3. Tumeh PC, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature.* 2014 Nov 27;515(7528):568-71.
4. D'Incecco A, *et al.* PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer.* 2015 Jan 6;112(1):95-102.
5. Tykodi SS. PD-1 as an emerging therapeutic target in renal cell carcinoma: current evidence. *Onco Targets Ther.* 2014 Jul 25;7:1349-59.
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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