PD-1 [CAL20]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3224-071318

BIOCARE MEDICAL

Catalog Number:	**ACI 3224 A, B**	**API 3224 AA**
Description:	0.1, 0.5, ml concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Renoir Red	N/A

Intended Use:
For In Vitro Diagnostic Use
PD-1 [CAL20] 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 member of the CD28/CTLA-4 family, and functions as a downregulator 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 (2,3). 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 melanoma, non-small-cell lung cancer, and renal cell carcinoma (3-5). This antibody can also be used in multiplex stains with other antibodies such as CD4, CD8, FOXP3, cytokeratin and melanoma markers (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, 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; others not tested
Clone: CAL20
Isotype: IgG1
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: Synthetic peptide derived from a region of the PD-1 protein
Cellular Localization: Cytoplasmic/cell membrane
Positive Tissue Control: Tonsil
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:
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protocol Recommendations Cont'd:
Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's 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 has been standardized with Biocare's MACH 4 detection system. Use TBS for washing steps.
Performance Characteristics:
Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.
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 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 in contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.

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Precautions Cont'd:
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. Yang G, *et al*. A multiplex IHC evaluation of multiple immune checkpoint receptors and mismatch repair proteins in colorectal carcinoma [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14-18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl):Abstract nr 1026.
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.

Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Breast Cancer	0	60
Colon Cancer	0	5
Lung Cancer	0	11
Prostate Cancer	0	10

Tumor-infiltrating lymphocytes (TILs) are positive in all cases when present.

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	0	3
Cerebellum	3	3
Adrenal	3	3
Ovary	0	2
Pancreas	0	3
Parathyroid	0	1
Pituitary	0	1
Testis	0	3
Thyroid	0	3
Breast	0	3
Spleen	3	3
Tonsil	3	3
Thymus	2	2
Bone Marrow	3	4
Lung	0	3
Heart	0	3
Esophagus	0	1
Stomach	0	3
Small Intestine	0	3
Colon	0	3
Liver	0	3
Salivary Gland	2	2
Kidney	3	3
Prostate	0	7
Uterus	0	3
Cervix	0	2
Skeletal Muscle	0	3
Skin	0	2
Peripheral Nerve	0	2
Linging Cells	0	2

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