PD-L1

Concentrated and Prediluted Rabbit Monoclonal Antibody
902-3171-111122

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 3171 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	APR 3171 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 3171 G	6.0 mL	Ready-to-use	N/A
Q-Series – For Leica BOND-III	ALR 3171 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.
Summary and Explanation:
Programmed death ligand 1 (PD-L1, also known as CD274) inhibits tumor-reactive T cells via binding to the programmed death-1 (PD-1) receptor, rendering tumor cells resistant to CD8+ T cell-mediated lysis.[1] Studies have shown that the inhibitory receptor PD-1 is expressed on tumor-infiltrating lymphocytes (TIL) while PD-L1 is expressed on tumor cells. Assessment of PD-L1 expression in combination with CD8+TIL density may be a useful predictive metric in multiple cancers, including stage III NSCLC, hormone receptor negative breast cancer and sentinel lymph node melanoma.[2-4] Clinical trials utilizing humanized chimeric antibodies that block inhibitory checkpoints, such as anti-PD-1 and anti-PD-L1, have demonstrated delayed tumor growth and increased survival.[5] While identification of PD-L1 overexpression by IHC is not yet standardized, it has become increasingly important to identify these tumors, as a directed therapy may improve clinical outcomes in these patients.[6] In cutaneous melanoma, the targeting of PD-1/PD-L1 has provided meaningful clinical benefit for patients in just the past 5-10 years.[7] The use of IHC for protein identification, along with novel therapies, such as checkpoint inhibitors and vaccines, are generating new options for the treatment of cancer patients. The PD-L1 [CAL10] clone does not cross react with PD-L2.
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: CAL10
Isotype: IgG
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: Peptide corresponding to the region within human PD-L1
Cellular Localization: Membranous/cytoplasmic
Positive Tissue Control: Lung adenocarcinoma or 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. 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.

Staining 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 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.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:
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 4 detection system. Use TBS for washing steps.
2. This clone demonstrates a weak protein expression in endothelial cells.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
AVR3171 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 64 minutes
Peroxidase: Pre-Primary Peroxidase Inhibitor
Primary Antibody: 60 minutes, 36°C

Staining Protocol Recommendations (Q Series – For Leica BOND-III):
ALR3171 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 + Blocker
Detection: Bond polymer Refine
HIER: 20 min with ER2
Peroxide Block: 5 min
Background Block: 10 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

Limitations:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.
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)[8]
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

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

Precautions Cont'd:
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.[9]
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.

Technical Support:
Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

References:
1. Ostrand-Rosenberg S, Horn LA, Haile ST. The programmed death-1 immune-suppressive pathway: barrier to antitumor immunity. J Immunol. 2014 Oct 15;193 (8):3835-41.
2. Tokito T, *et al*. Predictive relevance of PD-L1 expression combined with CD8+ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy. Eur J Cancer. 2016 Jan 6;55:7-14.
3. Park IH, *et al*. Prognostic implications of tumor-infiltrating lymphocytes in association with programmed death ligand 1 expression in early-stage breast cancer. Clin Breast Cancer. 2016 Feb;16(1):51-8.
4. Kakavand H, *et al*. Tumor PD-L1 expression, immune cell correlates and PD-1+ lymphocytes in sentinel lymph node melanoma metastases. Mod Pathol. 2015 Dec;28 (12):1535-44.
5. Xia B, Herbst RS. Immune checkpoint therapy for non-small-cell lung cancer: an update. Immunotherapy. 2016;8(3):279-98.
6. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther. 2015 Apr;14(4):847-56.
7. Singh BP, Salama AK. Updates in therapy for advanced melanoma. Cancers (Basel). 2016 Jan 15;8(1).
8. 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."
9. 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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