Recombinant Monoclonal Anti-PD-L1 Antibody, Rabbit (5D3), Ready-To-Use

Catalog Number: CAA-B001

Specification: 7.5 mL

IMPORTANT: Please carefully read this user guide before performing your experiment

Product Information

This IHC antibody reagent is intended for the *in vitro* semi-quantitative detection of PD-L1 protein status in neutral buffered formalin-fixed and paraffin-embedded (FFPE) tissue sections of non-small cell lung cancer, esophageal squamous cell carcinoma, gastric cancer, colorectal cancer, and hepatocellular carcinoma. It is used for histological evaluation.

The PD-L1 IHC assay employs a ready-to-use PD-L1 primary antibody (clone 5D3) to detect PD-L1 protein in FFPE tissue sections. Following antibody incubation, chromogenic visualization is performed using a ready-to-use enzymatic detection system. The enzymatic reaction with a subsequently added chromogen generates a visible reaction product at antigen sites. Tissue sections are then counterstained, dehydrated, cleared, and coverslipped. Results are evaluated under a light microscope.

Precautions:

1. Do not use this product after the expiration date indicated on the reagent label.
2. Do not mix or substitute reagents with those from different brands or sources.
3. This reagent is for research use only, not for diagnostic or therapeutic application.

Contact Information:

Manufactured and distributed by
ACRODiagnostics Inc.

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Content

Recombinant Monoclonal Anti-PD-L1 Antibody, Rabbit (5D3) with antibody diluent.

Storage

Stable for 12 months from the date of manufacture if stored at 2°C to 8°C, improper storage conditions will lead to test invalidation. Avoid freezing. Return the product to 2-8°C immediately after use.

Application Platform

LEICA BOND III

Required Materials (Not Supplied)

Instrument	LEICA BOND-III Fully Automated IHC and ISH Stainer
Reagents	BOND Epitope Retrieval Solution 2
	BOND Wash Solution 10X
	BOND Polymer Refine Detection DAB
	Deionized Water
Consumables	Microscope Slides
	Coverslips
	Permanent mounting medium and ancillary reagents required for mounting coverslips.

Tissue Sample Requirements

All tissue sample slides must be prepared and properly preserved for immunohistochemistry assay with standardized processes. The suggestion is to prepare samples with formalin and do regular paraffin embedding.

For instance, tissue samples should be prepared as 3-4mm thick sections, and fixed into 10% neutral buffered formalin at room temperature for 18-24 hours. Followingly, sample slides are dehydrated through a graded alcohol series (e.g., 70% to 100% ethanol), transparentized with xylene, then infiltrated with molten paraffin wax at a temperature below 60°C for embedding.

Tissue samples should be cut into 3-5μm thick and mounted on the glass slides. The slides for processed PD-L1 protein evaluation and tumor validation should be made simultaneously and stored at 2–8°C (prepared) or room temperature (≤25°C), avoid light until use.

Detection Method

Pre-experiment Notice

1. Before immunostaining, make sure CAA-B001 and other required reagents are all equilibrated to room temperature (18-27℃).
2. All incubation processes should be performed at room temperature (18-27℃).
3. Avoid slide drying during the staining procedures; otherwise, it may lead to increased non-specific background staining.

Reagent Preparation

BOND Wash Solution (1x washing buffer): Dilute 10x BOND Wash Solution Concentrate with 1:10 ratio to make sufficient BOND Wash Solution (1x washing buffer) with deionized water for IHC assay.

Notice: Avoid vigorous shaking or vortexing. Store aliquots at -70°C. Avoid repeated freeze-thaw cycles.

Operation Steps

1. **Dewaxing, Hydration and Antigen Retrieval**: After dewaxing and hydration, tissue section slides (FFPE) are immersed into BOND Epitope Retrieval Solution 2 (1x antigen retrieval solution). After incubate at 100℃ for 25 minutes, let tissue section slides cool down naturally to room temperature within the antigen retrieval solution. Wash the slides 5 times with BOND Wash Solution (1x washing buffer), then incubate at room temperature for 3 minutes.
2. **Blocking**: Incubate with inactivated endogenous peroxidase (Peroxide Block) at room temperature for

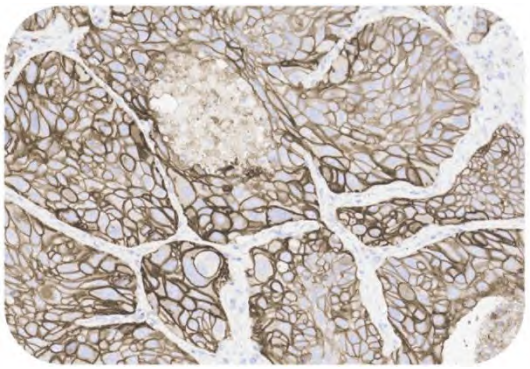
5 minutes and wash 3 times with BOND Wash Solution (1x washing buffer).

3. **Primary Antibody Incubation:** Add about 150 µL PD-L1 antibody reagent to each slide and incubate at room temperature for 35 minutes. Then wash the slides 3 times with BOND Wash Solution (1x washing buffer).
4. **Secondary Antibody Incubation:**
   a) Add about 150 µL Post Primary (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the slides 2 minutes for 3 times with BOND Wash Solution (1x washing buffer).
   b) Add about 150 µL Polymer (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the slides 2 minutes for 2 times with BOND Wash Solution (1x washing buffer), 2 minutes for once with deionized water.
5. **DAB Color Development**: Add about 150 µL Mixed DAB Refine (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 10 minutes, then wash the slides 3 times with deionized water.
6. **Counterstaining:** Stain each slide with about 150 µL hematoxylin at room temperature for 5 minutes, then wash the slides once with deionized water, once with BOND Wash Solution (1x washing buffer), then once with deionized water.
7. **Mounting**: After dehydration and transparency, use an appropriate amount of mounting medium to mount, observe the slides under a microscope and interpret the results.

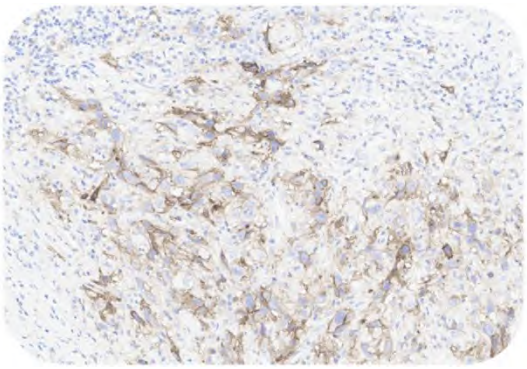
Typical Data

Cancer Sample & Indication Validation

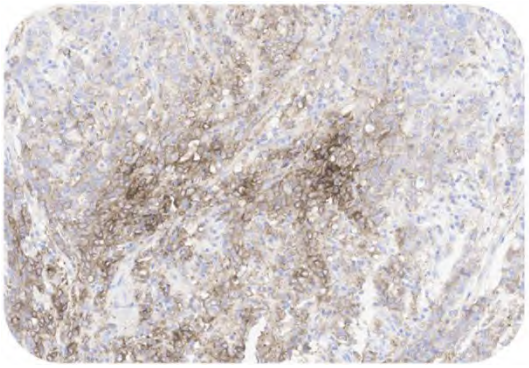
Use CAA-B001 to stain non-small cell lung cancer (NSCLC), esophageal squamous cell cancer, gastric cancer, colorectal cancer, and liver cancer samples. The results are showed below.



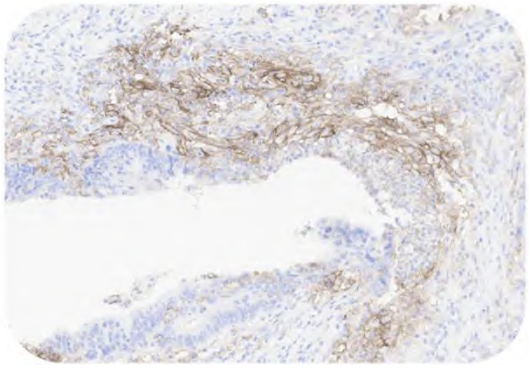
Human NSCLC Tissue – 20X



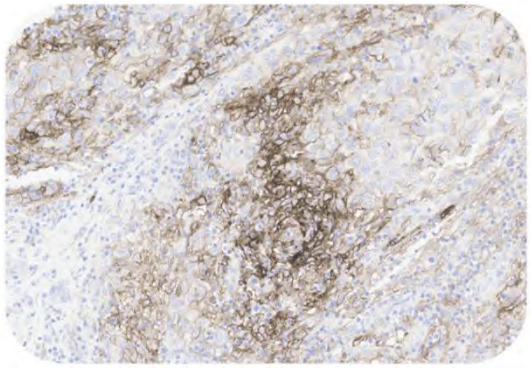
Human Esophageal Squamous Cell Cancer Tissue – 20X



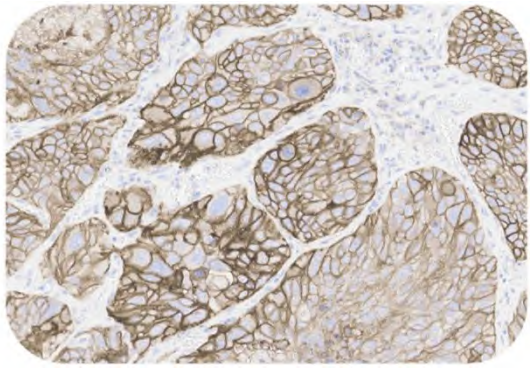
Human Gastric Cancer Tissue – 20X



Human Colorectal Cancer Tissue – 20X



Human Liver Cancer Tissue – 20X



Human NSCLC Tissue – 20X