

VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody

REF 790-4905

07494190001

IVD  50

INTENDED USE

VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the detection of the PD-L1 protein in formalin-fixed, paraffin-embedded tissue. It is intended to be stained with BenchMark IHC/ISH instruments. It is indicated as an aid in the assessment of PD-L1 expression in human tissues.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody (VENTANA PD-L1 (SP263) antibody) is a rabbit monoclonal primary antibody produced against programmed death-ligand 1 (PD-L1) also known as B7 homolog 1 (B7-H1) or CD274. It recognizes a transmembrane bound glycoprotein that has a molecular mass of 45-55 kDa. This antibody produces membranous and/or cytoplasmic staining.

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors programmed death-1 (PD-1) and B7-1 (CD80).¹ PD-1 is an inhibitory receptor expressed on T cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.² Binding of PD-L1 with PD-1 inhibits T cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T cells. CD80 is a molecule expressed on antigen presenting cells and activated T cells. PD-L1 binding to CD80 on T cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.³ PD-L1 expression has been observed in immune cells and tumor cells.^{4,5} Aberrant expression of PD-L1 on tumor cells and tumor associated immune cells has been reported to impede anti-tumor immunity, resulting in immune evasion.^{2,5} Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment.

PD-L1 is expressed in a broad range of cancers including lung, melanoma, urothelial, ovarian, and colorectal cancer. Prevalence of PD-L1 expression has been reported from 12% to 100% depending on the tumor type, anti PD-L1 clone and cutoff for positivity.⁶

PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP263) antibody is a rabbit monoclonal primary antibody which binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be localized using a haptened secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the BenchMark IHC/ISH instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies LCS (Cat. No. 650-210 / 05424534001 for BenchMark ULTRA and Cat. No. 650-010 / 05264839001 for BenchMark GX and XT), which minimizes evaporation of the aqueous reagents from the specimen slide.

In addition to staining with VENTANA PD-L1 (SP263) antibody, a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001). The negative reagent control is used to assess background staining.

REAGENT PROVIDED

VENTANA PD-L1 (SP263) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PD-L1 (SP263) antibody contains approximately 8.05 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 1.61 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA PD-L1 (SP263) antibody is a recombinant rabbit monoclonal antibody produced as cell purified culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

- Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
- Microscope slides, positively charged
- Bar code labels
- Xylene (Histological grade)
- Ethanol or reagent alcohol (Histological grade)
 - 100% solution: Undiluted ethanol or reagent alcohol
 - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
 - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
- Deionized or distilled water
- OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
- EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
- Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
- ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
- LCS (Predilute) (Cat. No. 650-010 / 05264839001)
- ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
- Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
- Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
- Bluing Reagent (Cat. No. 760-2037 / 05266769001)
- Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
- Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
- Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
- Light microscope
- Absorbent wipes.

STORAGE

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with OptiView DAB IHC Detection Kit and a BenchMark IHC/ISH instrument. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for a period of at least 6 hours up to 72 hours. Acceptable fixatives for use with VENTANA PD-L1 (SP263) antibody are Zinc Formalin and Z-5 fixatives when used with at least 6 hours of fixation time. Other fixatives, including 95% alcohol, AFA and PREFER fixative, are unacceptable for use with the VENTANA PD-L1 (SP263) antibody. The amount used is 15 to 20 times the volume of tissue. Fixation can be performed at room temperature (15-25°C).⁷ Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic (IVD) use.
- For professional use only.
- ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- Consult local and/or state authorities with regard to recommended method of disposal.
- For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a BenchMark IHC/ISH instrument in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for VENTANA PD-L1 (SP263) antibody with OptiView DAB IHC Detection Kit and BenchMark IHC/ISH instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes Standard
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	BenchMark GX instrument 16 minutes, 37°C BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 16 minutes, 36°C
OptiView HQ Linker	8 min (default)
OptiView HRP Multimer	8 min (default)
Counterstain	Hematoxylin II, 4 to 8 minutes
Post Counterstain	Bluing, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁸

POSITIVE TISSUE CONTROL

An example of positive control tissue for this antibody is human term placental tissue, which shows moderate to strong uniform staining of the membrane and weak to strong uniform staining of the cytoplasm of trophoblast-lineage cells. Placental stromal tissue and vasculature can be used for assessment of any background staining.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA PD-L1 (SP263) antibody is membranous and/or cytoplasmic staining of tumor cells. Immune cells demonstrate linear membranous, diffuse cytoplasmic, and/or punctate staining.

SPECIFIC LIMITATIONS

VENTANA PD-L1 (SP263) antibody has been optimized on BenchMark IHC/ISH instruments in combination with the OptiView DAB IHC Detection Kit at a 16 minute primary antibody incubation time.

Cold ischemia testing of VENTANA PD-L1 (SP263) antibody using a xenograft tissue model did not establish any conditions from hour zero to hour 24 that were not favorable with the assay.

Sections approximately 4-5 µm in thickness should be cut and mounted on positively charged slides.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 2 and Table 3 and in the Repeatability section.

Specificity

Table 2. Specificity of VENTANA PD-L1 (SP263) antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Adrenal gland	0/3*	Mesothelium	0/3†
Bladder	0/3	Myeloid (bone marrow)	0/4*,†
Breast	0/3	Nerve (sparse)	0/3
Cerebellum	0/3	Ovary	0/3
Cerebrum	0/3	Pancreas	0/3*
Cervix	0/3	Parathyroid gland	0/4
Colon	0/3†	Prostate	0/3
Endometrium	0/3	Salivary gland	0/3†
Esophagus	1/3*,†	Skeletal muscle	0/3
Heart	0/3	Skin	0/4‡
Hypophysis	0/3*,†	Spleen	0/3†
Intestine, small	0/3†	Stomach	0/3*,†
Kidney	0/3†	Testis	0/3
Larynx	0/3†	Thymus gland	0/3†
Liver	0/3	Thyroid	0/3*,†
Lung	0/3†	Tonsil	3/3†
Lymph node	0/3†		

Additional staining observed: * Cytoplasmic staining, † Immune cell staining, ‡ Melanocyte staining.

Sensitivity

Table 3. Sensitivity of VENTANA PD-L1 (SP263) antibody was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Origin	Pathology	# positive / total cases	
		Tumor Cells	Immune Cells
Cerebrum	Glioblastoma	0/1	1/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	1/1
Cerebrum	Oligodendroglioma	0/1	0/1
Ovary	Serous adenocarcinoma	0/1	1/1
Ovary	Adenocarcinoma	1/1	0/1
Pancreas	Islet cell carcinoma	0/1	0/1
Pancreas	Adenocarcinoma	0/1	1/1
Testis	Seminoma	0/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	1/1	0/1
Breast	Intraductal carcinoma	0/1	1/1
Breast	Invasive ductal carcinoma	0/2	0/2
Spleen	Diffuse B-cell lymphoma	0/1	1/1
Lung	Small cell undifferentiated carcinoma	1/1	1/1
Lung	Squamous cell carcinoma	1/1	1/1
Lung	Adenocarcinoma	0/1	0/1
Esophagus	Neuroendocrine carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	0/1	0/1
Stomach	Signet-ring cell carcinoma	0/1	0/1
Intestine	Adenocarcinoma	0/1	0/1
Intestine	Stromal sarcoma	0/1	0/1
Colon	Adenocarcinoma	0/1	1/1
Colon	Interstitialoma	0/1	0/1
Rectum	Adenocarcinoma	0/1	0/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	0/1	0/1
Kidney	Clear cell carcinoma	0/1	0/1
Prostate	Adenocarcinoma	0/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	0/1	0/1

Origin	Pathology	# positive / total cases	
		Tumor Cells	Immune Cells
Uterus	Clear cell carcinoma of endometrium	0/1	0/1
Uterine cervix	Squamous cell carcinoma	0/2	2/2
Striated muscle	Embryonal rhabdomyosarcoma	0/1	0/1
Rectum	Malignant melanoma	0/1	0/1
Skin	Basal cell carcinoma	0/1	0/1
Skin	Squamous cell carcinoma	0/1	0/1
Back	Neurofibroma	0/1	1/1
Retroperitoneal	Neuroblastoma	0/1	0/1
Abdominal cavity	Malignant mesothelioma	0/1	0/1
Mediastinum	Diffuse B-cell lymphoma	1/1	1/1
Lymph node	Hodgkin's lymphoma	1/1	1/1
Lymph node	Diffuse B-cell lymphoma	1/1	1/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1	1/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bone	Osteosarcoma	0/1	1/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Smooth muscle	Moderate malignant leiomyosarcoma	0/1	0/1
Bladder	Transitional cell carcinoma (bladder)	1/1	1/1

Repeatability

Repeatability studies for VENTANA PD-L1 (SP263) antibody were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark ULTRA instrument.
- Intra-platform reproducibility on the BenchMark XT, GX and ULTRA instruments.
- Inter-platform reproducibility between the BenchMark XT and GX instruments and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

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