

VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody

REF 790-4905

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IVD  50

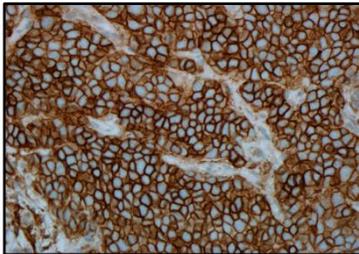


Figure 1. Non-small cell lung cancer stained with the VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody.

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 the VENTANA BenchMark ULTRA immunohistochemical automated slide stainer. It is indicated as an aid in the assessment of PD-L1 expression in non-small cell lung cancer (NSCLC) and other tumor types.

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) B7 homolog 1 (B7-H1, 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 (B7-H1, CD274) is part of a complex system of receptors and ligands that are involved in controlling T-cell activation. In normal tissue, PD-L1 is expressed on T cells, B cells, dendritic cells, macrophages, mesenchymal stem cells, bone marrow-derived mast cells, as well as various nonhematopoietic cells.¹ Its normal function is to regulate the balance between T-cell activation and tolerance through interaction with its 2 receptors, programmed death 1 (PD-1, CD279) and CD80 (B7-1). PD-L1 is also expressed by tumors and acts at multiple sites to help tumors evade detection and elimination by the host immune system. In the lymph nodes, PD-L1 on antigen presenting cells (APC) binding to PD-1 (CD279) or CD80 (B7-1) on activated T cells delivers an inhibitory signal to the T cell.^{1,2} Likewise, binding of CD80 on APCs to PD-L1 on T cells leads to inhibitory signaling in the T cell. These and bidirectional interactions between CD80 and PD-L1, expressed on both APCs and T cells, lead to further inhibition of T-cell activation and fewer activated T cells in the circulation. In the tumor microenvironment, PD-L1 expressed on tumor cells binds to PD-1 on activated T cells reaching the tumor. This delivers an inhibitory signal to those T cells, preventing them from killing target tumor cells, and protecting the tumor from immune elimination.³ PD-L1 is expressed in a broad range of cancers.

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). 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 VENTANA BenchMark ULTRA automated slide stainer washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies ULTRA LCS (ULTRA LCS (Predilute), Cat. No. 650-210), 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). 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:

1. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795)
2. Microscope slides, positively charged
3. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
4. Xylene (Histological grade)
5. 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
6. Deionized or distilled water
7. OptiView DAB IHC Detection Kit (Cat. No. 760-700)
8. EZ Prep Concentrate (10X) (Cat. No. 950-102)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300)
10. ULTRA LCS (Predilute) (Cat. No. 650-210)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224)
12. Hematoxylin II counterstain (Cat. No. 790-2208)
13. Bluing Reagent (Cat. No. 760-2037)
14. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
15. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
16. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
17. Light microscope
18. 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 the VENTANA OptiView DAB IHC detection kit and a VENTANA BenchMark ULTRA automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for a period of at least 6 hours up to 48 hours.⁴

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, are unacceptable for use with 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).⁵ 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

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. 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.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. 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 the VENTANA BenchMark ULTRA automated slide stainer 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 ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes Standard
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	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."⁶

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 VENTANA BenchMark ULTRA 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
Cerebrum	0/3	Myeloid (bone marrow)	0/2
Cerebellum	0/3	Lung	3/5
Adrenal gland	0/3	Heart	0/3
Ovary	0/3	Esophagus	0/3
Pancreas	0/3	Stomach	0/3
Hypophysis	0/3	Small intestine	1/3
Testis	0/3	Colon	2/3
Thyroid	0/3	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	3/3	Kidney	0/3
Tonsil	3/3	Prostate	0/3
Uterus	0/3	Cervix	0/3
Skeletal muscle	0/3	Skin	0/2
Nerve (sparse)	0/3	Lymph node	3/3
Thymus	3/3	Cardiac pericardium	0/1

Sensitivity

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

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical Meningioma	0/1
Malignant Ependymoma	0/1
Malignant Oligodendroglioma	0/1
Serous Adenocarcinoma	0/1

Pathology	# positive / total cases
Ovarian Adenocarcinoma	0/1
Islet cell tumor	1/1
Pancreatic Adenocarcinoma	0/1
Seminoma	0/1
Embryonal Carcinoma	0/1
Medullary Carcinoma	0/1
Papillary Carcinoma	1/1
Breast intraductal carcinoma	0/1
Breast invasive ductal carcinoma	0/2
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	1/1
Lung squamous cell carcinoma	1/1
Lung adenocarcinoma	0/1
Esophageal neuroendocrine carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric signet-ring cell carcinoma	0/1
Gastrointestinal adenocarcinoma	0/1
GIST	0/1
Colon adenocarcinoma	0/1
Colon interstitialoma	0/1
Rectal adenocarcinoma	0/1
Rectal interstitialoma	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostate adenocarcinoma	0/2
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	1/1
Uterine squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Rectal malignant melanoma	0/1
Basal cell carcinoma (skin)	0/1
Squamous cell carcinoma (skin)	0/1
Neurofibroma (back)	0/1
Retroperitoneal neuroblastoma	0/1
Malignant mesothelioma (abdominal cavity)	0/1

Pathology	# positive / total cases
Diffuse B-cell lymphoma (mediastinum)	0/1
Hodgkin lymphoma	1/1
Diffuse B-cell lymphoma (lymph node)	0/1
Anaplastic large cell lymphoma (pelvic cavity)	0/1
Low grade leiomyosarcoma (bladder)	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma (retroperitoneum)	0/1
Intermediate grade leiomyosarcoma (smooth muscle)	0/1
Transitional cell carcinoma (bladder)	0/1

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 ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

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