VENTANA PD-L1 (SP263) Rabbit Monoclonal Primary Antibody

REF 790-4905

07494190001

IVD Σ 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 (FFPE) 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 haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate. 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, 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.

MATERIAL 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 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.

Specific antibody concentration is approximately 1.6 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 method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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 method sheet 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. Recommended control tissue
2. Microscope slides, positively charged
3. Bar code labels
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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
8. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
9. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
10. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
11. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001) for BenchMark ULTRA and BenchMark ULTRA PLUS instruments
12. LCS (Predilute) (Cat. No. 650-010 / 05264839001) for BenchMark XT and BenchMark GX instruments
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
15. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
16. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
17. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
18. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
19. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
20. Light microscope
21. Absorbent wipes.

STORAGE AND STABILITY

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 FFPE 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

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. 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.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[8,9]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
WARNING 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, a reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

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 2 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 method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4905

Table 2. Recommended staining protocol for VENTANA PD-L1 (SP263) antibody with OptiView DAB IHC Detection Kit and BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX or XT	ULTRA or ULTRA PLUS
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes Standard	ULTRA Cell Conditioning 1, 64 minutes Standard
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
OptiView HQ Linker	8 min (default)	
OptiView HRP Multimer	8 min (default)	
Counterstain	Hematoxylin II, 4 or 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."[10]

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.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed below.

Sensitivity and Specificity

Arrays containing a variety of normal tissues were stained with VENTANA PD-L1 (SP263) antibody and evaluated for presence of membranous PD-L1 staining as listed in Table 3. Additional staining, such as cytoplasmic or immune cell staining, is also noted (see Table 3 footnote).

An array containing a variety of neoplastic tissues was stained with VENTANA PD-L1 (SP263) antibody evaluated for tumor cell and immune cell staining as listed in Table 4.

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Table 3. Sensitivity/specificity of VENTANA PD-L1 (SP263) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Myeloid (bone marrow) [a,b]	0/4
Cerebellum	0/3	Lung [b]	0/3
Adrenal gland [a]	0/3	Heart	0/3
Ovary	0/3	Esophagus [a,b]	1/3
Pancreas[a]	0/3	Stomach [a,b]	0/3
Parathyroid gland	0/4	Small intestine [b]	0/3
Pituitary gland [a,b]	0/3	Colon [b]	0/3
Testis	0/3	Liver	0/3
Thyroid [a,b]	0/3	Salivary gland [b]	0/3
Breast	0/3	Lymph node [b]	0/3
Spleen [b]	0/3	Kidney [b]	0/3
Larynx [b]	0/3	Prostate	0/3
Tonsil [b]	3/3	Cervix	0/3
Endometrium	0/3	Bladder	0/3
Skeletal muscle	0/3	Skin [c]	0/4
Nerve (sparse)	0/3	Mesothelium [b]	0/3
Thymus gland [b]	0/3		

Additional staining observed: [a] Cytoplasmic staining, [b] Immune cell staining, [c] Melanocyte staining.

Percent of immune cells present above background cannot be evaluated in this study because there is no tumor area for which to score tumor infiltrating immune cells.

Table 4. Sensitivity/Specificity of VENTANA PD-L1 (SP263) Assay was determined by testing a variety of FFPE neoplastic tissues for any tumor cell membranous and immune cell staining.

Pathology	# positive / total cases	
	Tumor Cells	Immune Cells
Glioblastoma (Cerebrum)	0/1	1/1
Meningioma (Cerebrum)	0/1	0/1
Ependymoma (Cerebrum)	0/1	1/1
Oligodendroglioma (Cerebrum)	0/1	0/1
Serous adenocarcinoma (Ovary)	0/1	1/1
Adenocarcinoma (Ovary)	1/1	0/1
Neuroendocrine neoplasm (Pancreas)	0/1	0/1
Adenocarcinoma (Pancreas)	0/1	1/1
Seminoma (Testis)	0/1	0/1
Embryonal carcinoma (Testis)	0/1	0/1
Medullary carcinoma (Thyroid)	0/1	0/1
Papillary carcinoma (Thyroid)	1/1	0/1
Ductal carcinoma in situ (Breast)	0/1	1/1
Invasive ductal carcinoma (Breast)	0/2	0/2
B-cell lymphoma, NOS (Spleen)	0/1	1/1
Small cell carcinoma (Lung)	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	1/1
Adenocarcinoma (Lung)	0/1	0/1
Neuroendocrine carcinoma (Esophagus)	0/1	0/1
Adenocarcinoma (Esophagus)	0/1	0/1
Signet-ring cell carcinoma (Stomach)	0/1	0/1
Adenocarcinoma (Small Intestine)	0/1	0/1
Stromal sarcoma (Small Intestine)	0/1	0/1
Adenocarcinoma (Colon)	0/1	1/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1	0/1
Adenocarcinoma (Rectum)	0/1	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1	0/1
Hepatocellular carcinoma (Liver)	0/1	0/1
Hepatoblastoma (Liver)	0/1	0/1
Clear cell carcinoma (Kidney)	0/1	0/1
Adenocarcinoma (Prostate)	0/2	0/2
Leiomyoma (Uterus)	0/1	0/1
Adenocarcinoma (Uterus)	0/1	0/1
Clear cell carcinoma (Uterus)	1/1	0/1
Squamous cell carcinoma (Cervix)	0/2	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1	0/1
Melanoma (Rectum)	0/1	0/1
Basal cell carcinoma (Skin)	0/1	0/1
Squamous cell carcinoma (Skin)	0/1	0/1
Neurofibroma (Back)	0/1	1/1
Neuroblastoma (Retroperitoneum)	0/1	0/1
Mesothelioma (Abdominal cavity)	0/1	0/1
B-cell lymphoma, NOS (Mediastinum)	1/1	1/1
Hodgkin lymphoma (Lymph node)	1/1	1/1
B-cell lymphoma, NOS (Lymph node)	1/1	1/1
Anaplastic large cell lymphoma (Pelvic cavity)	1/1	1/1
Leiomyosarcoma (Bladder)	0/1	0/1
Osteosarcoma (Bone)	0/1	1/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1	0/1
Leiomyosarcoma (Smooth muscle)	0/1	0/1
Urothelial 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.
- Method Comparison on BenchMark ULTRA PLUS vs BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
D	Added BenchMark ULTRA PLUS instrument. Updates to Principle of the Procedure, Materials Required But Not Provided, Warnings and Precautions, Staining Procedure, and Performance Characteristics sections. Removed CE mark.

INTELLECTUAL PROPERTY

For USA: Rx only

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