VENTANA PTEN (SP218) Rabbit Monoclonal Primary Antibody

REF 790-5097

07970200001

IVD Σ 50

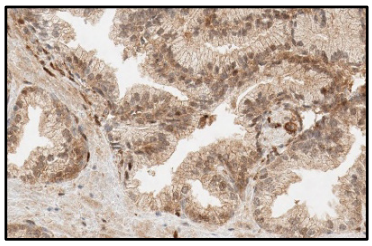

Figure 1. Prostate tissue stained with VENTANA PTEN (SP218) antibody.

INTENDED USE

VENTANA PTEN (SP218) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of the phosphatase and tensin homolog (PTEN) protein by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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

The VENTANA PTEN (SP218) Rabbit Monoclonal Primary Antibody (VENTANA PTEN (SP218) antibody) is directed against the dual specificity protein phosphatase encoded by the tumor suppressor gene phosphatase and tensin homolog (PTEN) at chromosome 10q23.3.[1,2] PTEN mediated dephosphorylation is important because it results in inhibition of the Akt signaling pathway, which plays an important role in controlling cell survival and cell cycle progression.[3,4] The PTEN protein is localized in the cytoplasm and nucleus, and is an essential enzyme expressed in several non-neoplastic tissues.[1,5] Reduced or absent PTEN protein expression has been identified by immunohistochemical analyses in several cancers, including prostate, breast, thyroid, endometrial, gastric, non-small cell lung, and pancreatic.[6-12]

PTEN is one of the most commonly lost tumor suppressors in prostate cancer.[13,14] The loss of PTEN protein expression in prostate cancer is frequently associated with advanced disease.[15,16] The assessment of PTEN protein expression by immunohistochemistry (IHC) may be used to aid in the characterization of an advanced phenotype in prostate cancer.[13-16] Although PTEN protein loss is correlated with clinicopathological features of advanced prostate cancer, it does not provide independent prognostic information.[13,15] Results of a PTEN IHC assay are intended to provide supplemental information regarding the tumor phenotype and are to be interpreted in the context of established prognostic indicators.

PRINCIPLE OF THE PROCEDURE

VENTANA PTEN (SP218) antibody binds to PTEN protein in sections of formalin-fixed, paraffin-embedded (FFPE) samples. The specific antibody can be localized using a haptenated 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. Refer to the OptiView DAB IHC Detection Kit method sheet for further information.

MATERIAL PROVIDED

VENTANA PTEN (SP218) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PTEN (SP218) antibody contains approximately 15 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris buffered saline, EDTA, Brij-35 with carrier protein and sodium azide, a preservative.

Specific antibody concentration is approximately 3 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA PTEN (SP218) antibody is a Protein A purified recombinant rabbit monoclonal antibody.

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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. General purpose laboratory equipment
14. BenchMark IHC/ISH instrument

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 VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[17] Sections should be cut at approximately 3-6 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is sodium azide. Symptoms of overexposure to sodium azide include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of sodium azide in this product is 0.05% and does not meet the criteria for a hazardous substance. Build-up of 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 accumulation in plumbing.[18] Systemic allergic reactions are possible in sensitive individuals.

6. 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.
7. 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.[19,20]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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 dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with the 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 instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding IHC staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-5097.

Table 1. Recommended staining protocol VENTANA PTEN (SP218) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 56 minutes	CC1, 56 minutes	ULTRA CC1 56 minutes, 100°C
Pre antibody peroxidase inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 37°C	16 minutes, 37°C
OptiView HQ Linker	12 minutes	12 minutes	8 minutes (default)
OptiView HRP Multimer	12 minutes	12 minutes	8 minutes (default)
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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, or cell conditioning pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[21]

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA PTEN (SP218) antibody, a second slide should be stained with Rabbit Monoclonal Negative Control Ig.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of positive control tissue for this antibody is normal pancreas, which demonstrates staining of the cytoplasm and/or nucleus in islet cells and acinar cells. The associated non-neoplastic stromal cells, endothelial cells, or peripheral nerve cells within the tumor region are examples of positive internal controls.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA PTEN (SP218) antibody is cytoplasmic and/or nuclear. Examples of expected staining results are shown in Figure 2 and Figure 3.

SPECIFIC LIMITATIONS

Slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slide stability within their own environment when storing beyond 45 days.

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 sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 2. Sensitivity/Specificity of VENTANA PTEN (SP218) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Breast	2/2	Nerve	2/2
Cerebellum	1/1	Ovary	3/3
Cerebrum	2/2	Prostate	1/1
Cervix	1/1	Skeletal Muscle	0/3
Colon	3/3	Skin	1/1
Eye	1/1	Small intestine	3/3
Kidney	1/1	Spleen	1/1
Larynx	3/3	Stomach	2/2
Liver	2/2	Tongue	2/2
Lung	2/2	Uterus	2/2

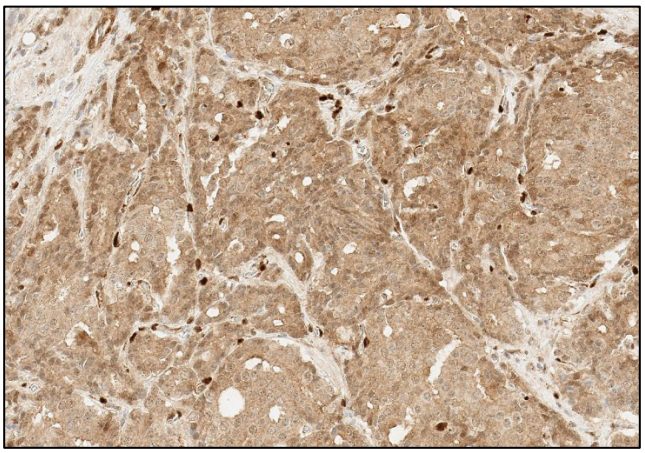

Figure 2. Presence of VENTANA PTEN (SP218) antibody staining in prostate cancer.

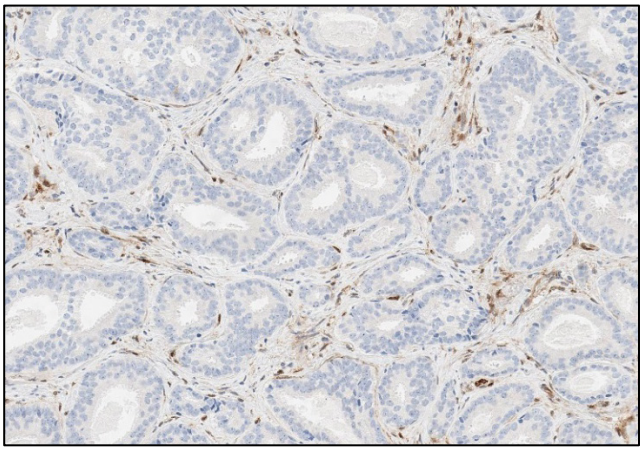

Figure 3. Absence of VENTANA PTEN (SP218) antibody staining in prostate cancer.

Table 3. Sensitivity/Specificity of VENTANA PTEN (SP218) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma [a]	1/1
Meningioma	0/1
Ependymoma	1/1
Oligodendroglioma	0/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	1/1
Adenocarcinoma (Pancreas) [a]	1/1
Seminoma (Testis)	0/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Intraductal carcinoma (Breast)	1/1
Invasive ductal carcinoma (Breast) [b]	49/64
Diffuse B-cell lymphoma (Spleen)	0/1
Papillary adenocarcinoma	1/2
Adenocarcinoma (Lung) [c]	22/30
Carcinoma (sparse) (Lung)	1/1
Large cell carcinoma (Lung)	3/5
Mucinous adenocarcinoma (Lung)	1/4
Solid mucinous cell carcinoma (Lung)	3/3
Small cell undifferentiated carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung) [d]	20/40
Neuroendocrine carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Signet-ring cell carcinoma [a]	1/1
Stromal sarcoma	1/1
Adenocarcinoma (Colorectal) [a]	2/2
Gastrointestinal Stromal Tumor	1/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate) [e]	46/66
Squamous cell carcinoma (Cervix)	1/1
Embryonal rhabdomyosarcoma	0/1
Squamous cell carcinoma (Skin) [a]	1/1
Neuroblastoma	0/1
Mesothelioma [a]	1/1
Diffuse B cell lymphoma	2/2
Mixed type Hodgkin lymphoma	0/1
Urothelial carcinoma (Bladder)	1/1
Osteosarcoma	0/1
Leiomyosarcoma	0/1

[a] focal staining in all positive cases
[b] focal staining in 3/49 positive cases
[c] focal staining in 2/22 positive cases
[d] focal staining in 2/20 positive cases
[e] focal staining in 10/46 positive cases

Precision

Precision studies for VENTANA PTEN (SP218) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark ULTRA, BenchMark XT and BenchMark GX instruments.
- Between platform precision between the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria

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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.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
B	Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Materials Required but not Provided, Storage and Stability, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Staining Interpretation / Expected Results, Specific Limitations, Analytical Performance, Clinical Performance, References, Symbols, Intellectual Property, and Contact Information sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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