

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody

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IVD Σ 50

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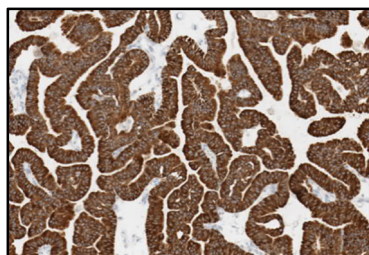


Figure 1. VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody-stained NSCLC tissue.

INTENDED USE

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative detection of ROS1 protein in formalin-fixed, paraffin-embedded tissue stained with VENTANA BenchMark IHC/ISH instruments. 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 ROS1 (SP384) Rabbit Monoclonal Primary Antibody is a rabbit monoclonal antibody produced against the ROS1 protein, a receptor tyrosine kinase of the insulin receptor family. The ROS1 gene is activated by chromosomal rearrangements. These chromosomal rearrangements result in a portion of ROS1, including the kinase domain, fused with 1 of 27 currently known partner genes.¹ The resulting overexpression of ROS1 has been demonstrated to promote tumorigenesis in a variety of cancers.²⁻⁶

PRINCIPLE OF THE PROCEDURE

The VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody binds to ROS1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. 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 package insert for further information.

In addition to staining with VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody, a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001).

REAGENT PROVIDED

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody contains approximately 0.8 µg of a rabbit monoclonal antibody.

The antibody is diluted in a Tris-HCl diluent with 10 mg/mL of carrier protein, ProClin 300 preservative and 0.17% Brij-35.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.16 µg/mL.

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert 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 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. 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. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
6. Bluing Reagent (Cat. No. 760-2037 / 05266769001)

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 VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.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. 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. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any IHC assay (for example, lack of primary antibody or counterstain on the tissue). Ask your Roche representative for a copy of "Impacts of Environmental Stresses on IHC Positively Charged Slides" to better understand how to use these types of slides.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. Consult local and/or state authorities with regard to recommended method of disposal.
9. 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 BenchMark IHC/ISH instruments 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 ROS1 (SP384) Rabbit Monoclonal Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments. The staining procedure OptiView DAB IHC should be used.

Procedure Type	Method
	GX, XT, and ULTRA
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	CC1 64 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	16 minutes, 36°C or 37°C ^a
OptiView HQ Linker	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

^a 36°C on ULTRA, 37°C on GX and XT

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

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.

Examples of positive control tissues for this antibody are known ROS1 positive NSCLC tissues, reactive alveolar type II pneumocytes, and cells lines known to express ROS1-fusions such as HCC78.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody is mainly cytoplasmic and rarely membranous and nuclear.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on the BenchMark IHC/ISH instrument in combination with the OptiView DAB IHC Detection Kit using the parameters listed in Table 1, but the user must validate results obtained with this reagent. All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

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 Precision section.

Sensitivity and Specificity

Table 2. Sensitivity/Specificity of VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	1/8 ^a	Adrenal gland	0/5
Cerebellum	1/4 ^a	Ovary	0/5
Pancreas	1/6 ^b	Stomach	1/5 ^e
Lymph node	0/5	Small intestine	2/5 ^f
Hypophysis	0/6	Colon	0/6
Testis	2/6 ^c	Liver	0/6
Thyroid gland	3/6 ^d	Salivary gland	0/6
Breast	0/3	Kidney	5/5 ^g
Spleen	0/5	Prostate	2/5 ^h
Tonsil	0/5	Endometrium	0/3
Thymus gland	0/5	Cervix	0/3
Parathyroid	0/3	Skeletal muscle	0/4
Bladder	0/4	Skin	1/12 ⁱ
Bone marrow	0/5	Nerve	0/4
Lung	0/16	Mesothelium	3/4 ^j
Heart	1/3	Eye	0/1
Esophagus	0/6	Larynx	0/1

^a Neural cytoplasmic staining; ^b Acinar/islet cytoplasmic staining;

^c Stromal staining; ^d Colloid staining; ^e Focal luminal secretion staining;

^f Lymphocyte staining in stroma; ^g Apical membrane staining;

^h Apical membrane and epithelial cytoplasmic staining;

ⁱ Epidermal cytoplasmic and lymphocyte staining;

^j Type II pneumocyte staining in one case

Table 3. Sensitivity/Specificity of VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Tissue	Pathology	# positive / total cases
Cerebrum	Glioblastoma	0/2
Cerebrum	Atypical meningioma	0/1
Cerebrum	Oligodendroglioma	0/1
Ovary	High grade serous carcinoma	1/2 ^a
Pancreas	Islet cell tumor	0/1
Pancreas	Duct adenocarcinoma	0/1
Testis	Seminoma	0/1
Testis	Embryonal carcinoma	0/1
Thyroid	Medullary carcinoma	0/1
Thyroid	Papillary carcinoma	0/1
Breast	Intraductal carcinoma with early infiltrate	1/1 ^b
Breast	Invasive ductal carcinoma	0/2
Spleen	Diffuse B-cell lymphoma	0/1
Lung	Small cell undifferentiated carcinoma	1/1 ^a
Lung	Squamous cell carcinoma	3/27
Lung	Adenocarcinoma	4/15
Lung	Papillary adenocarcinoma	1/2
Lung	Mucinous adenocarcinoma	0/2
Lung	Adenosquamous carcinoma	0/1
Esophagus	Squamous cell carcinoma	0/1
Esophagus	Adenocarcinoma	0/1
Stomach	Mucinous adenocarcinoma	0/1
Small intestine	Adenocarcinoma	0/1
Small intestine	Malignant interstitialoma	0/1
Colon	Adenocarcinoma	0/1
Colon	Moderate malignant interstitial sarcoma	0/1
Rectum	Adenocarcinoma	0/1
Rectum	Malignant interstitialoma	0/1
Liver	Hepatocellular carcinoma	0/1
Liver	Hepatoblastoma	0/1
Liver	Intrahepatic cholangiocarcinoma	12/89

Tissue	Pathology	# positive / total cases
Kidney	Clear cell carcinoma	0/1
Prostate	Adenocarcinoma	0/2
Uterus	Lowly malignant leiomyosarcoma	0/1
Uterus	Adenocarcinoma	0/1
Uterus	Clear cell carcinoma	0/1
Cervix	Squamous cell carcinoma	0/1
Cervix	Invasive squamous cell carcinoma	0/1
Striated muscle	Embryonal rhabdomyosarcoma	0/1
Rectum	Malignant melanoma	0/1
Skin	Basal cell carcinoma	0/1
Skin	Squamous cell carcinoma	0/1
Skin	Malignant melanoma	2/40
Retroperitoneum	Neuroblastoma	0/1
Peritoneum	Malignant mesothelioma	1/1 ^a
Lymph node	Diffuse B cell lymphoma	0/2
Lymph node	Hodgkin's lymphoma	1/1 ^c
Bladder	High grade urothelial carcinoma	0/1
Bladder	Leiomyosarcoma	0/1
Peritoneum	Highly malignant pleomorphic rhabdomyosarcoma	0/1

^a Lymphocyte staining; ^b Cytoplasmic staining; ^c Staining of Hodgkin's cells

Precision

Precision studies for VENTANA ROS1 (SP384) Rabbit Monoclonal Primary 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 GX, BenchMark XT, and BenchMark ULTRA instruments.
- Between platform precision between the BenchMark GX, BenchMark XT, and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

IHC AND FISH COMPARISON DATA

Table 4 shows the results of an analytical comparison study of cases that were stained with VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody (IHC) and were also tested with fluorescence in situ hybridization (FISH).

Table 4. VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody analytical comparison data – IHC vs. FISH

IHC	FISH Result			Agreement Rate % ^a (n/N) (95% CI) ^b
	Positive	Negative	Total	
≥ 2+ staining in cytoplasm in any of tumor cells	45	18	63	PPA: 97.8 (45/46) (88.7, 99.6)
≥ 2+ staining in cytoplasm not in any of tumor cells	1	56	57	NPA: 75.7 (56/74) (64.8, 84.0)
Total	46	74	120	OPA: 84.2 (101/120) (76.6, 89.6)
≥ 2+ staining in cytoplasm in > 25% of total tumor cells	45	11	56	PPA: 97.8 (45/46) (88.7, 99.6)
≥ 2+ staining in cytoplasm in ≤ 25% of total tumor cells	1	63	64	NPA: 85.1 (63/74) (75.3, 91.5)
Total	46	74	120	OPA: 90.0 (108/120) (83.3, 94.2)
≥ 2+ staining in cytoplasm in > 30% of total tumor cells	45	8	53	PPA: 97.8 (45/46) (88.7, 99.6)
≥ 2+ staining in cytoplasm in ≤ 30% of total tumor cells	1	66	67	NPA: 89.2 (66/74) (80.1, 94.4)
Total	46	74	120	OPA: 92.5 (111/120) (86.4, 96.0)
≥ 2+ staining in cytoplasm in > 50% of total tumor cells	42	5	47	PPA: 91.3 (42/46) (79.7, 96.6)
≥ 2+ staining in cytoplasm in ≤ 50% of total tumor cells	4	69	73	NPA: 93.2 (69/74) (85.1, 97.1)
Total	46	74	120	OPA: 92.5 (111/120) (86.4, 96.0)

^a PPA = positive percent agreement; NPA = negative percent agreement; OPA = overall percent agreement;

^b Two-sided 95% confidence interval using the Wilson Score method; CI = Confidence Interval

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