VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody

REF 741-7130

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IVD Σ 50

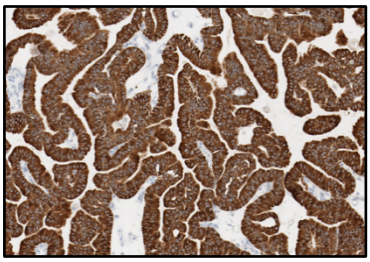

Figure 1. VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody-stained NSCLC tissue.

INTENDED USE

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of ROS1 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

VENTANA ROS1 (SP384) Rabbit Monoclonal Primary Antibody (VENTANA ROS1 (SP384) antibody) is a rabbit monoclonal antibody produced against the ROS1 protein, a receptor tyrosine kinase of the insulin receptor family.[1-5] The ROS1 gene is susceptible to chromosomal rearrangements.[1-5] These chromosomal rearrangements result in a portion of ROS1, including the kinase domain, to be fused with one of several currently known partner genes.[1-6] The resulting overexpression of these novel ROS1 rearranged proteins has been demonstrated to promote tumorigenesis in a variety of cancers including non-small cell lung cancer (NSCLC).[1-6] ROS1 immunohistochemistry (IHC) can detect wild-type and rearranged ROS1 proteins and thus determination of *ROS1* gene rearrangement status is not possible by ROS1 IHC alone.[1-5] Therefore, tissues that exhibit ROS1 expression by IHC should be further characterized by a molecular and/or cytogenetic method.[7,8,9]

The detection of ROS1 protein by IHC with the VENTANA ROS1 (SP384) antibody may be used to aid in the identification of non-small cell lung cancer (NSCLC) with ROS1 expression for further characterization by a molecular and/or cytogenetic method. The staining pattern is mainly cytoplasmic and rarely membranous and nuclear.

PRINCIPLE OF THE PROCEDURE

VENTANA ROS1 (SP384) 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 respective method sheet for further information.

MATERIAL PROVIDED

VENTANA ROS1 (SP384) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA ROS1 (SP384) antibody contains approximately 1 µg of a rabbit monoclonal antibody.

The antibody is diluted in in a Tris-HCl diluent with carrier protein, 0.10% ProClin 300 preservative and 0.17% Brij-35.

Specific antibody concentration is approximately 0.2 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA ROS1 (SP384) antibody is a recombinant rabbit monoclonal antibody produced as purified cell 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. 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.[10] Sections should be cut at approximately 4 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. 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.[11,12]
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 BenchMark IHC/ISH instruments 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 instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

Table 2. Recommended staining protocol for VENTANA ROS1 (SP384) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments. Use OptiView DAB IHC staining procedure.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes,	CC1, 64 minutes,	ULTRA CC1, 64 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37 °C	16 minutes, 37 °C	16 minutes, 36 °C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	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, 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".[13]

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA ROS1 (SP384) 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.

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) antibody is mainly cytoplasmic and rarely membranous and nuclear. Evaluation criteria are based on the pathologist's expertise on how to interpret the staining results of the assay and to determine if the test specimen should undergo further testing using other molecular and/or cytogenetic methods. The guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology recommends that all ROS1 IHC positive results undergo confirmation by FISH or a molecular method.[9] The guideline also state that current published evidence is insufficient to recommend one specific scoring system to define ROS1 positivity and each laboratory must validate its own interpretive staining criteria from known positive and negative samples.[9]

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 2, 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

ANALYTICAL PERFORMANCE

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

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Sensitivity and Specificity

Table 3. Sensitivity/Specificity of VENTANA ROS1 (SP384) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum[a]	1/8	Adrenal gland	0/5
Cerebellum[a]	1/4	Ovary	0/5
Pancreas[b]	1/6	Stomach[e]	1/5
Lymph node	0/5	Small intestine[f]	2/5
Pituitary gland	0/6	Colon	0/6
Testis[c]	2/6	Liver	0/6
Thyroid gland[d]	3/6	Salivary gland	0/6
Breast	0/3	Kidney[g]	5/5
Spleen	0/5	Prostate[h]	2/5
Tonsil	0/5	Endometrium	0/3
Thymus gland	0/5	Cervix	0/3
Parathyroid gland	0/3	Skeletal muscle	0/4
Bladder	0/4	Skin[i]	1/12
Myeloid (bone marrow)	0/5	Nerve	0/4
Lung	0/16	Mesothelium[j]	3/4
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 4. Sensitivity/Specificity of VENTANA ROS1 (SP384) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/2
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)[a]	1/2
Neuroendocrine neoplasm (Pancreas)	0/1
Duct adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Intraductal carcinoma (Breast)[b]	1/1
Invasive ductal carcinoma (Breast)	0/2
Diffuse B-cell lymphoma (Spleen)	0/1
Small cell carcinoma (Lung)[a]	1/1
Squamous cell carcinoma (Lung)	3/27
Adenocarcinoma (Lung)	4/15
Papillary adenocarcinoma (Lung)	1/2
Mucinous adenocarcinoma (Lung)	0/2
Adenosquamous carcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Gastrointestinal)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Intrahepatic cholangiocarcinoma (Liver)	12/89
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyosarcoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/1
Invasive squamous cell carcinoma (Cervix)	0/1
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Melanoma (Rectum)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Melanoma (Skin)	2/40
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)[a]	1/1
B-Cell Lymphoma; NOS (Lymph node)	0/2
Hodgkin lymphoma (Lymph node)[c]	1/1
High grade urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Spindle cell rhabdomyosarcoma (Peritoneum)	0/1

[a] Lymphocyte staining; [b] Cytoplasmic staining; [c] Staining of Hodgkin cells

Precision

Precision studies for VENTANA ROS1 (SP384) 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.

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.

IHC and FISH Comparison Data

Table 5 shows the results of an analytical comparison study of cases that were stained with VENTANA ROS1 (SP384) antibody for IHC and were also tested with fluorescence in situ hybridization (FISH). The analytical comparison data for the VENTANA ROS1 (SP384) antibody versus FISH are provided for informational purposes only. All assay and evaluation criteria must be validated by the clinical laboratory in its own setting, taking into account pre-analytics and other factors unique to each laboratory setting.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of VENTANA ROS1 (SP384) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

Table 5. VENTANA ROS1 (SP384) 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	58	59	NPA: 76.3 (58/76) (65.6, 84.5)
Total	46	76	122	OPA: 84.4 (103/122) (77.0, 89.8)
≥ 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	65	66	NPA: 85.5 (65/76) (75.9, 91.7)
Total	46	76	122	OPA: 90.2 (110/122) (83.6, 94.3)
≥ 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	68	69	NPA: 89.5 (68/76) (80.6, 94.6)
Total	46	76	122	OPA: 92.6 (113/122) (86.6, 96.1)
≥ 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	71	75	NPA: 93.4 (71/76) (85.5, 97.2)
Total	46	76	122	OPA: 92.6 (113/122) (86.6, 96.1)

[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

REFERENCES

1. Rossi G, Jocolle G, Conti A, et al. Detection of ROS1 Rearrangement in Non-Small Cell Lung Cancer: Current and Future Perspectives. Lung Cancer (Auckl). 2017;8:45-55.
2. Bubendorf L, Buttner R, Al-Dayel F, et al. Testing for ROS1 in Non-Small Cell Lung Cancer: A Review with Recommendations. Virchows Arch. 2016;469(5):489-503.
3. Gainor JF, Shaw AT. Novel Targets in Non-Small Cell Lung Cancer: ROS1 and RET Fusions. Oncologist. 2013;18(7):865-875.
4. Lin JJ, Shaw AT. Recent Advances in Targeting ROS1 in Lung Cancer. J Thorac Oncol. 2017;12(11):1611-1625.
5. Davies KD, Doebele RC. Molecular Pathways: ROS1 Fusion Proteins in Cancer. Clin Cancer Res. 2013;19(15):4040-4045.
6. Varella-Garcia M, Yoshida A. ROS1 Testing with FISH. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. Ed. Tsao M, Ed. Hirsch F, Ed. Yatabe Y. 2nd Edition. Aurora, CO, USA. Editorial Rx Press; 2016. 55-61. Print.
7. D. Planchard, S. Popat, K. Kerr, et al. ESMO Clinical Practice Metastatic NSCLC Guidelines for Diagnosis, Treatment and Follow Up. Ann Oncol. 2019.
8. Wu YL, Planchard D, Lu S, et al. Pan-Asian Adapted Clinical Practice Guidelines for the Management of Patients with Metastatic Non-Small-Cell Lung Cancer: A CSCO-ESMO Initiative Endorsed by JSMO, KSMO, MOS, SSO and TOS. Ann Oncol. 2019;30(2):171-210.
9. Lindeman NI, Cagle PT, Aisner DL, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment with Targeted Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med. 2018;142(3):321-346.
10. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
11. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
12. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
13. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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 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
C	Updates to Warnings and Precautions. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

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