VENTANA MSLN (SP74) ASSAY

REF 790-7028

08508011001

IVD Σ 50

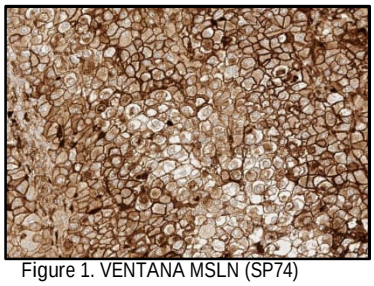

Figure 1. VENTANA MSLN (SP74) Assay expression on mesothelioma tissue.

INTENDED USE

VENTANA MSLN (SP74) Assay is intended for the immunohistochemical assessment of the mesothelin protein in formalin-fixed, paraffin-embedded (FFPE) tissue stained with 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 MSLN (SP74) Assay is a rabbit monoclonal antibody produced against the mesothelin (MSLN) protein. The VENTANA MSLN (SP74) Assay antibody demonstrates cytoplasmic and membranous staining.

Mesothelin (MSLN) is a 40-kDa membrane bound cell surface glycoprotein that is largely restricted to normal mesothelial cells lining the pleura, pericardium, and peritoneum.[1-4] The biological function of MSLN is unknown, though it has been observed that the protein has limited distribution on normal tissues and high expression in pancreatic cancer, ovarian cancer, mesothelioma, and other cancers.[5]

PRINCIPLE OF THE PROCEDURE

VENTANA MSLN (SP74) Assay is a rabbit monoclonal primary antibody that binds to MSLN in paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 0639500001). Refer to the OptiView DAB IHC Detection Kit package insert for further information.

In addition to staining with VENTANA MSLN (SP74) Assay, a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001).

MATERIAL PROVIDED

VENTANA MSLN (SP74) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA MSLN (SP74) Assay contains approximately 5 µg of a rabbit monoclonal antibody.

The antibody is diluted in phosphate buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein.

Specific antibody concentration is approximately 1 µg/mL.

VENTANA MSLN (SP74) Assay is a Protein A purified recombinant rabbit monoclonal antibody.

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 the OptiView DAB IHC Detection Kit 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. Human normal fallopian tube specimens for use as a control tissue
2. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
3. Microscope slides, positively charged
4. Bar code labels
5. Xylene (Histological grade)
6. 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
7. Deionized or distilled water
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. LCS (Predilute) (Cat. No. 650-010 / 05264839001) for BenchMark XT and GX automated staining instruments
12. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001) for BenchMark ULTRA automated staining instrument
13. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001) for BenchMark XT and GX automated staining instruments
14. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001) for BenchMark ULTRA automated staining instrument
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

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 OptiView DAB Detection Kit and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin for 6 to 72 hours.[6] Fixatives with high concentration of alcohol (e.g. 95% ethanol) or glyoxal-based fixatives (e.g. PREFER fixative) should be avoided. 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 navifyportal.roche.com.

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.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	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 the OptiView DAB IHC 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 package insert for more details regarding immunohistochemistry staining procedures.

Table 2. Recommended staining protocol for VENTANA MSLN (SP74) Assay with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected		
Cell Conditioning (Antigen Unmasking)	CC1 72 minutes, 100 °C	CC1 64 minutes, 100 °C	ULTRA CC1 64 minutes, 100 °C
Antibody (Primary)	8 minutes, 37 °C	8 minutes, 37 °C	8 minutes, 36 °C
OptiView HQ Linker	8 minutes		
OptiView HRP Multimer	8 minutes		
Counterstain	Hematoxylin II, 4 to 12 minutes		
Post Counterstain	Bluing, 4 to 12 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 based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[7]

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 a control tissue for this antibody is normal fallopian tube tissue, which demonstrates weak to strong luminal membrane staining in the epithelium, and absence of specific staining in the stroma. Preferred staining for an acceptable system-level control is weak to moderate staining in the epithelium and absence of specific staining in the stroma of the normal fallopian tube. The epithelial staining is used as an internal positive control and the absence of specific staining in the stroma is used as an internal negative control.

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by VENTANA MSLN (SP74) Assay.

The cellular staining pattern for VENTANA MSLN (SP74) Assay is membranous and cytoplasmic in neoplastic tissue. Stromal staining in high-expressing cases is common and should not be considered unacceptable background when present.

SPECIFIC LIMITATIONS

Histological samples should be fixed within 24 hours of collection with 10% neutral buffered formalin for 6-72 hours.

Tissue sections approximately 4 µm in thickness should be cut and mounted on positively charged slides.

Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.

Tissue staining is dependent on the handling and processing of the tissue before staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.

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 3 and Table 4 and in the Precision section.

Sensitivity and Specificity

Table 3. Specificity of VENTANA MSLN (SP74) Assay was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Bone marrow	0/3
Cerebellum	0/3	Lung	0/3
Adrenal gland	0/3	Pericardium/ mesothelium	3/3
Ovary	2/3	Cardiac muscle	0/3
Endometrium	1/3	Esophagus	0/3
Cervix	1/3	Stomach	0/3
Pancreas	0/3	Small intestine	2/3
Lymph Node	0/3	Colon	3/3
Hypophysis	0/3	Liver	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Testis	0/3	Salivary gland	0/3
Parathyroid gland	0/3	Kidney	0/3
Thyroid gland	0/4	Bladder	3/3
Breast	0/3	Prostate	0/3
Spleen	0/5	Skeletal muscle	0/4
Tonsil	2/3	Skin	0/3
Thymus gland	3/3	Peripheral nerve	0/3

Table 4. Sensitivity of VENTANA MSLN (SP74) Assay was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
B-cell Lymphoma; NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small Intestine)	0/1
Adenocarcinoma (Colon)	1/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Melanoma (Rectum)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	1/1
B-Cell Lymphoma; NOS (Lymph node)	0/2
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma (Peritoneum)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

Precision

Precision studies for VENTANA MSLN (SP74) Assay were completed to demonstrate:

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

All studies met their acceptance criteria.

REFERENCES

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4. Chowdhury PS, Viner JL, Beers R, Pastan I. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. Proc Natl Acad Sci USA. 1998;95(2):669-74.
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6. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
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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
B	Updates to Material Provided and Warnings and Precautions sections. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

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