INSM1 (SP493) Rabbit Monoclonal Primary Antibody

REF 790-7247

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IVD Σ 50

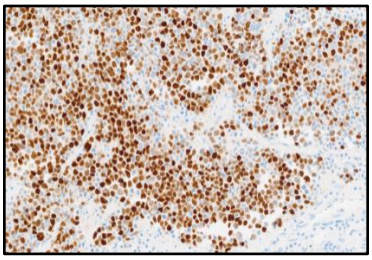

Figure 1. INSM1 (SP493) antibody staining of small cell lung carcinoma.

INTENDED USE

INSM1 (SP493) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of INSM1 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

INSM1 (SP493) Rabbit Monoclonal Primary Antibody (INSM1 (SP493) antibody) is a rabbit monoclonal antibody designed for the detection of insulinoma-associated protein 1 (INSM1) protein. The primary structure of INSM1 has five zinc-finger DNA binding motifs and dibasic amino acid conversion sites.[1,2] INSM1 is known to coordinate the differentiation of developing neuroendocrine tissues through a variety of transcriptional networks.[1]

Neuroendocrine neoplasms (NENs) are rare neoplasms that originate in neuroendocrine cells. The types of NENs include well-differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs), and NENs of neuronal type which are known as pheochromocytoma (PHEO) / paraganglioma (PGL).[3,4] When diagnosing a NEN, pathologists rely on confirmation of neuroendocrine differentiation of the lesion, which is often accomplished by immunohistochemical detection of neuroendocrine biomarkers.[3,4] NETs usually express biomarkers of neuroendocrine differentiation such as. synaptophysin and chromogranin A, as well as INSM1.[4] In contrast, NECs have shown varied positivity with synaptophysin and chromogranin A, but INSM1 has emerged as a useful neuroendocrine marker.[3] Expression of INSM1, synaptophysin, and chromogranin A is often expressed diffusely in PHEOs and PGLs.[5]

The detection of INSM1 protein by immunohistochemistry (IHC) with the INSM1 (SP493) antibody may be used to aid in the identification of neuroendocrine neoplasms.

PRINCIPLE OF THE PROCEDURE

INSM1 (SP493) antibody binds to the INSM1 antigen in formalin-fixed paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No 760-700 / 063965000001) and *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

INSM1 (SP493) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of INSM1 (SP493) antibody contains approximately 25 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris buffered saline, EDTA, Brij-35, and 0.05% sodium azide, a preservative. There is trace amount of bovine serum albumin, a carrier protein.

Specific antibody concentration is approximately 5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

INSM1 (SP493) 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. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. Amplification Kit (Cat. No. 760-080 / 05266114001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. General purpose laboratory equipment
16. 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 formalin-fixed, paraffin-embedded (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.[6] 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. 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.
5. 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.[7,8]
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. 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.
9. Consult local and/or state authorities with regard to recommended method of disposal.

10. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
11. 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 VENTANA detection kits and accessories. Refer to the tables below 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.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-7247.

Table 1. Recommended staining protocol for INSM1 (SP493) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	
Cell Conditioning (Antigen Unmasking)	CC1, 72 minutes	ULTRA CC1, 64 minutes, 100 °C
Pre-Primary Peroxidase Inhibitor	Selected	
Antibody (Primary)	32 minutes, 37 °C	16 minutes, 36 °C
OptiView HQ Linker	8 minutes	
OptiView HRP Multimer	8 minutes	
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.

Table 2. Recommended staining protocol for INSM1 (SP493) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1, 64 minutes, 95 °C
Antibody (Primary)	32 minutes, 37 °C	32 minutes, 36 °C
Amplification	Selected	
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."[9]

NEGATIVE REAGENT CONTROL

In addition to staining with INSM1 (SP493) antibody, a second slide should be stained with the appropriate negative control reagent.

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 normal pancreas with positive INSM1 staining in islet cells.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for INSM1 (SP493) antibody is nuclear.

SPECIFIC LIMITATIONS

OptiView DAB IHC Detection Kit is generally more sensitive than *ultra*View Universal DAB Detection Kit. The user must validate the results obtained with this reagent and detection systems.

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 3. Sensitivity/Specificity of INSM1 (SP493) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/4	Colon [h]	0/4
Cerebellum	0/4	Liver	0/4
Adrenal gland [a]	2/6	Salivary gland	0/3
Ovary	0/4	Kidney	0/6
Pancreas [b]	23/23	Prostate	0/4
Lymph node [c]	1/1	Bladder	0/4
Parathyroid gland	0/3	Endometrium	0/4
Pituitary gland [d]	2/4	Cervix	0/4
Testis	0/4	Skeletal muscle	0/3
Thyroid [e]	0/18	Skin	0/3
Breast	0/4	Nerve	0/3
Spleen [f]	1/3	Mesothelium	0/3
Tonsil [c]	2/3	Eye	0/1
Thymus [g]	3/3	Fallopian tube	0/3
Bone marrow	0/3	Rectum [h]	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Lung	0/16	Ureter	0/2
Heart	0/3	Placenta	0/3
Esophagus	0/3	Spinal cord	0/2
Stomach [h]	0/4		
Small intestine [h]	0/4		

[a] medulla chromaffin cells stained positive

[b] includes normal and benign pancreas; islet cells are positive with nuclear staining

[c] subset of lymphocytes stained positive with nuclear staining

[d] glandular cells are positive with nuclear staining

[e] includes normal, thyroiditis, and goiter tissues

[f] few positive cells that could be a subset of lymphocytes

[g] subset of lymphocytes and epithelial cells stained positive with nuclear staining

[h] scattered normal neuroendocrine cells stained positive

Table 4. Sensitivity/Specificity of INSM1 (SP493) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Meningioma (Cerebellum)	0/2
Meningioma (Brain)	0/1
Astrocytoma (Brain)	0/1
Adenoma (Pituitary gland)	1/1
Adenocarcinoma (Head and Neck)	0/1
Melanoma (Head and Neck)	0/1
Squamous cell carcinoma (Head and Neck)	0/1
Nasopharyngeal carcinoma (Nasopharynx)	0/1
Pleomorphic adenoma (Salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Endometrioid adenocarcinoma (Ovary)	0/1
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Acinar cell carcinoma (Pancreas) [a]	1/1
Adenocarcinoma (Pancreas)	0/1
Ductal adenocarcinoma (Pancreas) [b,c]	0/10
Adenosquamous carcinoma (Pancreas)	0/1
Islet cell tumor (Pancreas) [a]	1/2
Mixed duct-neuroendocrine carcinoma (Pancreas)	1/1
Mucinous adenocarcinoma (Pancreas)	0/1
Neuroendocrine carcinoma (Pancreas)	1/3
Pleomorphic carcinoma (Pancreas) [b]	0/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/5
Follicular adenoma (Thyroid)	0/2
Clear cell carcinoma (Thyroid)	0/2
Follicular carcinoma (Thyroid)	0/3
Papillary adenocarcinoma (Thyroid)	0/3
Undifferentiated carcinoma (Thyroid)	0/2
Adenoma (Parathyroid gland)	0/4
Fibroadenoma (Breast)	0/2
Invasive ductal carcinoma (Breast)	0/3
Papillary adenocarcinoma (Lung) [a]	1/8
Adenocarcinoma (Lung) [a]	1/28
Adenocarcinoma in situ (Lung)	0/8
Giant cell carcinoma (Lung)	0/2
Large cell carcinoma (Lung)	0/7
Squamous cell carcinoma (Lung)	0/28
Atypical carcinoid (Lung)	6/8
Neuroendocrine carcinoma (Lung)	1/1
Small cell carcinoma (Lung)	88/88
Metastatic adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/3
Adenocarcinoma (Stomach)	0/3
Adenoma (Small intestine) [d]	0/1
Adenocarcinoma (Small intestine) [d]	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	1/3
Carcinoid (Intestine, rectum)	2/2
Adenocarcinoma (Rectum) [d]	0/3
Hepatocellular carcinoma (Liver)	0/4
Metastatic colon adenocarcinoma (Liver) [a]	1/1
Clear cell carcinoma (Kidney)	0/2
Adenoma, cortical (Adrenal gland)	0/2
Adrenocortical carcinoma (Adrenal gland)	0/1
Pheochromocytoma (Adrenal gland)	3/3
Adenocarcinoma, cortical (Adrenal gland)	0/1
Adenocarcinoma (Prostate) [a]	1/2
Squamous cell carcinoma (Cervix)	0/2
Adenocarcinoma (Endometrium)	0/2
Squamous cell carcinoma (Skin)	0/1
B-Cell Lymphoma; NOS (Lymph node)	0/1
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Metastatic breast ductal carcinoma (Lymph node)	0/1
Metastatic esophageal squamous cell carcinoma (Lymph node)	0/1
Metastatic lung adenocarcinoma (Lymph node)	0/8

Pathology	# positive / total cases
Metastatic lung squamous cell carcinoma (Lymph node) [a]	2/4
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Neuroendocrine carcinoma (Scalp) [a]	1/1

[a] <1% tumor cells stained positive

[b] normal islet cells with moderate to strong nuclear staining

[c] subset of lymphocytes stained positive

[d] scattered normal neuroendocrine cells stained positive

Precision

Precision studies for INSM1 (SP493) 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 ULTRA / BenchMark ULTRA PLUS instrument.
- Between platform precision between the BenchMark GX and BenchMark ULTRA / BenchMark ULTRA PLUS instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included within run, between day, and between instrument precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of INSM1 (SP493) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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.

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.

INTELLECTUAL PROPERTY

For USA: Rx only

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