CONFIRM anti-S100 (Polyclonal) Primary Antibody

REF 760-2523

05267072001

IVD Σ 50

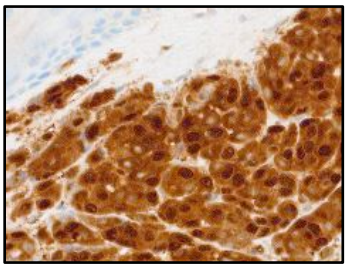

Figure 1. CONFIRM anti-S100 (Polyclonal) Primary Antibody exhibiting a nuclear and cytoplasmic staining pattern in melanoma tissue.

INTENDED USE

CONFIRM anti-S100 (Polyclonal) Primary Antibody is a rabbit polyclonal antibody intended for laboratory use in the qualitative immunohistochemical detection of S100 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 S100 protein family consists of at least 25 low molecular weight (9–13 kDa) calcium-binding EF-hand proteins.[1] The majority of these proteins are homo- and heterodimers in which the monomers are non-covalently bound.[1] Members of the S100 family are expressed in a wide variety of cell types and are involved in regulating diverse intracellular processes such as contraction, motility, cell growth, cell cycle regulation, regulation of transcription factors, and protein phosphorylation.[1,2] Select S100 proteins are also secreted and/or released by cellular damage and have extracellular functions.[1,2]

S100 is expressed in several cell types including melanocytes, astrocytes, Langerhans cells, cells of cartilaginous and adipose tissue, glial and neural cells, Schwann cells, and myoepithelial cells.[1,3] Neoplasms derived from these cell types also express S100, such as melanomas, selected histiocytic proliferations, schwannomas, various carcinomas (e.g., salivary gland carcinomas, sweat gland carcinomas), gliomas, and peripheral nerve sheath tumors (PNST).[1,3]

Detection of S100 protein by immunohistochemistry (IHC) with CONFIRM anti-S100 (Polyclonal) Primary Antibody (CONFIRM anti-S100 (Polyclonal) antibody) may be used as a melanocyte marker to aid in the differential diagnosis of melanocytic versus non-melanocytic tumors. It may be used as part of a panel of IHC studies. The staining pattern is cytoplasmic and nuclear.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-S100 (Polyclonal) antibody is a rabbit polyclonal antibody produced against purified bovine brain S100 protein. CONFIRM anti-S100 (Polyclonal) antibody binds to the S100 protein in formalin-fixed, paraffin-embedded tissue (FFPE) sections and exhibits a nuclear and cytoplasmic staining pattern. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001), *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001), or *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-S100 (Polyclonal) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-S100 (Polyclonal) antibody contains approximately 50 µg of a rabbit polyclonal antibody.

The antibody is diluted in a phosphate buffered saline containing carrier protein and 0.05% ProClin 300 as a preservative.

Specific antibody concentration is approximately 10 µg/mL. There is no known non-specific antibody reactivity observed in this product.

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. CONFIRM Negative Control Rabbit Ig (Cat. No. 760-1029 / 05266238001)
4. VENTANA Antibody Diluent with Casein (Cat. No. 760-219 / 06440002001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
7. *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001)
8. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
10. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
11. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
12. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
15. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
16. Permanent mounting medium
17. Cover glass
18. Automated coverslipper
19. General purpose laboratory equipment
20. 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 series of automated instruments. The recommended tissue fixative is 10% neutral buffered formalin.[4] 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. 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. 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.

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.[5,6]
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.

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 dust/ fume/ gas/ mist/ vapours/ spray.
	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, 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 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 760-2523.

Table 2. Recommended staining protocol for CONFIRM anti-S100 (Polyclonal) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	ULTRA CC1, Mild
Antibody (Primary)	16 minutes, 37°C	24 minutes, 36°C
ultraBlock [b]	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.

[b] Use of VENTANA Antibody Diluent with Casein at the ultraBlock step.

Table 3. Recommended staining protocol for CONFIRM anti-S100 (Polyclonal) antibody with *ultra*View Universal Alkaline Phosphatase Red Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	ULTRA CC1, Mild
Antibody (Primary)	16 minutes, 37°C	24 minutes, 36°C
ultraBlock [b]	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.

[b] Use of VENTANA Antibody Diluent with Casein at the ultraBlock step.

Table 4. Recommended staining protocol for CONFIRM anti-S100 (Polyclonal) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1 16 minutes	ULTRA CC1 16 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	12 minutes, 37°C	12 minutes, 36°C
Option 2 [b]	8 minutes	
OptiView HQ Linker	8 minutes (default)	
OptiView HRP Multimer	8 minutes (default)	

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
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.
[b] Option 2 use of VENTANA Antibody Diluent with Casein.

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."[7]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-S100 (Polyclonal) 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.

The recommended positive control tissue is appendix. Schwann cells in peripheral nerve fibers, ganglionic satellite cells in the muscularis propria and submucosa should be strongly positive as should adipocytes and dendritic cells and macrophages in the lamina propria. Epithelial cells should be negative.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-S100 (Polyclonal) antibody is nuclear and cytoplasmic.

SPECIFIC LIMITATIONS

OptiView detection system is generally more sensitive than other detection systems. 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 5. Sensitivity/Specificity of CONFIRM anti-S100 (Polyclonal) antibody was determined by testing FFPE normal tissues.

Tissue [a]	# positive / total cases	Tissue	# positive / total cases
Cerebrum [b]	3/3	Colon	0/3
Cerebellum [b]	6/6	Rectum	0/3
Brain [b]	4/4	Liver	0/4
Adrenal gland [c,d]	1/4	Salivary gland [h]	5/5
Ovary	0/4	Kidney	0/6
Pancreas [e]	3/4	Prostate [c]	0/4
Parathyroid gland	0/5	Bladder	0/5
Pituitary gland [f]	3/3	Ureter	0/2
Testis [g]	3/4	Endometrium	0/5
Thyroid	0/4	Fallopian tube	0/3
Breast [h]	3/4	Placenta	0/3
Spleen [i]	4/4	Cervix	0/4
Tonsil [j]	4/4	Skeletal muscle	0/3
Thymus [j]	3/3	Skin [k]	10/10
Bone marrow [i]	1/3	Nerve [l]	5/5
Lung	0/4	Spinal cord [b]	2/2
Heart	0/4	Mesothelium	0/3
Esophagus	0/4	Soft Tissue [l,m]	1/1
Stomach	0/4	Lymph Node [i,j]	0/1
Small intestine	0/4		

[a] S100 is expressed in many normal structures such as histiocytes, dendritic cells, adipocytes, nerve and myoepithelial cells. When evaluating the above normal tissues, a positive or negative status was derived based on assessment of the site-specific normal cells; therefore, tissues deemed as negative may have staining in some or all of the aforementioned structures., [b] Neurons and glial cells, [c] Tissue evaluated includes normal and hyperplasia., [d] Medullary cells, [e] Islet cells, [f] Folliculo-stellate cells, [g] Leydig cells, [h] Myoepithelial cells, [i] Histiocytes, [j] Dendritic cells, [k] Melanocytes, [l] Schwann cells, [m] Adipocytes

Table 6. Sensitivity/Specificity of CONFIRM anti-S100 (Polyclonal) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology [a]	# positive / total cases
Astrocytoma (Brain)	36/38
Glioblastoma (Brain)	4/5
Oligodendroglioma	5/5
Ependymoma (Brain)	2/2
Medulloblastoma (Cerebellum)	1/1
Meningioma (Brain)	3/4
Atypical meningioma (Brain)	1/2
Meningioma, fibroblastic (Brain)	6/7
Meningioma, meningothelial (Brain)	0/2
Meningioma, psammomatous (Brain)	3/4
Meningioma, transitional (Brain)	1/2
Anaplastic meningioma (Brain)	2/6

Pathology [a]	# positive / total cases
Schwannoma (Brain)	3/3
Hemangioblastoma (Cerebellum)	0/1
CNS embryonal tumor (Brain)	1/1
Craniopharyngioma	1/1
Neuroblastoma	0/3
Metastatic carcinoma (Brain)	0/10
Adenocarcinoma (Head and neck)	1/1
Squamous cell carcinoma (Head and neck)	0/1
Adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	1/1
Granulosa cell tumor (Ovary)	1/1
Adenocarcinoma (Ovary)	0/2
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/3
Papillary adenocarcinoma (Thyroid)	0/1
Fibroadenoma (Breast)	2/2
Invasive ductal carcinoma (Breast)	6/21
Invasive lobular carcinoma (Breast)	0/5
Metastatic breast ductal carcinoma (Lymph node)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	0/1
Metastatic cancer (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/3
Metastatic esophagus squamous cell carcinoma (Lymph node)	0/1
Adenocarcinoma (Stomach)	0/2
Adenoma (Small intestine)	0/1
Adenocarcinoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/4
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Metastatic colon adenocarcinoma (Liver)	0/1
Adenocarcinoma (Rectum)	0/3
Hepatocellular carcinoma (Liver)	0/4
Pleomorphic adenoma (Head and neck, salivary gland)	1/1
Adenoid cystic carcinoma (Head and neck, salivary gland)	1/1

Pathology [a]	# positive / total cases
Clear cell carcinoma (Kidney)	0/2
Adenocarcinoma (Prostate)	0/2
Squamous cell carcinoma (Cervix)	0/2
Adenocarcinoma (Endometrium)	0/2
Squamous cell carcinoma (Skin)	0/6
Basal cell carcinoma (Skin)	0/5
Melanoma	38/39
Metastatic melanoma	38/39
Nevus (Skin)	7/7
Neurofibroma (Skin)	6/6
B-Cell Lymphoma; NOS	0/2
Hodgkin lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	0/2
Osteosarcoma	0/6
Chondrosarcoma	3/4
Giant cell tumor (Bone)	1/9
Ameloblastoma (Head and neck)	0/2
Metastatic carcinoma (Bone)	0/4
Lipoma (Trunk)	1/1
Liposarcoma	13/16
Fibroma	0/2
Fibrosarcoma	1/20
Dermatofibrosarcoma protuberans	0/3
Undifferentiated / unclassified sarcoma	0/4
Rhabdomyosarcoma (Peritoneal cavity)	1/1
Rhabdomyosarcoma, embryonal	1/3
Rhabdomyosarcoma, polymorphic	2/3
Rhabdomyosarcoma, alveolar	1/3
Leiomyosarcoma	0/8
Synovial sarcoma	0/3
Epithelioid sarcoma (Hand)	0/2
Spindle cell sarcoma (Peritoneal cavity)	0/1
Clear cell sarcoma (Leg)	1/1
Carcinosarcoma (Peritoneal cavity)	0/1
Malignant peripheral nerve sheath tumor (MPNST)	12/20

[a] S100 is expressed in many normal structures such as histiocytes, dendritic cells, adipocytes, nerve and myoepithelial cells. When evaluating the above tumors, a positive

or negative status was derived based on assessment of the tumor cells; therefore, tumors deemed as negative may have staining in some or all of the aforementioned structures.

Precision

Precision studies for CONFIRM anti-S100 (Polyclonal) 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, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, 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.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-S100 (Polyclonal) 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 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
G	Updates to Image, Staining Procedure and Analytical Performance sections. Removed XT and updated Sensitivity and Specificity tables.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE 0123