CONFIRM anti-S100 (Polyclonal) Primary Antibody

REF 760-2523

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IVD Σ 50

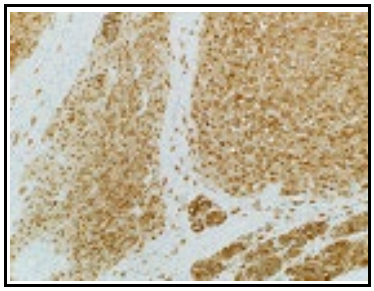

Figure 1. CONFIRM anti-S100 (polyclonal) cytoplasmic staining of melanoma.

INTENDED USE

Ventana Medical Systems, Inc.'s (Ventana) CONFIRM anti-S100 (Polyclonal) Primary Antibody (CONFIRM anti-S100 (Polyclonal)) contains rabbit antiserum directed against an epitope found on S100 protein and may be used to aid in the identification of cells of normal and abnormal neuronal and neuroendocrine lineage and as an aid in the diagnosis of anaplastic tumors. The antibody is intended for qualitative staining in section of formalin-fixed, paraffin-embedded tissue on a VENTANA automated slide stainer.

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

S100 is a 20 kD acidic calcium binding protein named for its partial solubility in saturated ammonium sulfate. It is composed of two subunits that can be one of two types, alpha and beta. These subunits are expressed differently by a variety of individual human tissues.[1]

S100 is a sensitive marker for melanoma. It stains amelanotic melanomas more strongly than pigmented tumors and detects melanomas that are often negative for other melanocytic markers such as desmoplastic melanomas. The staining is cytoplasmic, the proportion of positive cells varying from 10 to 70 percent or more.[1]

Although S100 is highly sensitive for melanomas, many non melanocytic tumors also show S100 positivity. S100 typically is present in neural cells (glial cells and Schwann cells) and their corresponding tumors.[1] An advantage of the ubiquity of strongly immunoreactive S100 protein in peripheral nerves, which are present in almost any section of normal or diseased tissue, is that it provides a built in positive control for most immunostains for S100.[2]

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-S100 Rabbit (Polyclonal) may be used as the primary antibody for immunohistochemical staining of paraffin tissue sections. In general, immunohistochemical staining allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and cover slipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

CONFIRM anti-S100 Rabbit (Polyclonal) is optimally diluted for use with VENTANA detection kits and automated slide stainers. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the VENTANA automated slide stainer to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen containing slide a coverslip solution is applied in the slide stainer. Staining is completed after incubation with a substrate chromogen and optional counterstaining. For more detailed information on instrument operation, refer to the appropriate VENTANA automated slide stainer operator's manual.

REAGENT PROVIDED

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

One dispenser of CONFIRM anti-S100 Rabbit (Polyclonal) contains 5 mL of prediluted reagent. The dispenser contains approximately 50 µg/mL (10 µg/mL) of rabbit polyclonal antiserum directed against human S100 protein. The antiserum is diluted in 0.1 M phosphate buffered saline containing a carrier protein and ProClin 300, a preservative. Total protein concentration of the reagent is approximately 3 mg/mL.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) 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.

STORAGE

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 a VENTANA BenchMark XT automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[3] 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. ProClin 300 is used as a preservative in this solution. 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.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a VENTANA BenchMark XT automated slide stainer in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocol.

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's 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 CONFIRM anti-S100 Rabbit (Polyclonal) Primary Antibody with *i*VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Enhancement)	None required,
Antibody (Primary)	BenchMark XT instrument 16 minutes
Counterstain	Hematoxylin, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes

POSITIVE TISSUE CONTROL

A tissue with weak positive staining is more suitable for optimal quality control. Examples of types of tissue to use as weak positive control with CONFIRM anti-S100 (Polyclonal) are peripheral nerve, intestine or adrenal. The positive staining cells and tissue components (Schwann cells), are used to confirm that the antibody was applied and the instrument functioned properly.

STAINING INTERPRETATION / EXPECTED RESULTS

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

SPECIFIC LIMITATIONS

Unexpected antigen expression or loss of expression may occur, especially in neoplasms. The occasional positively stained dendritic cell in the interstitial lung tissue between the alveoli could either correspond to an interdigitating reticulum cell or a Langerhans cell.[4] Occasionally stromal elements surrounding heavily stained tissue and or cells will show apparent immunoreactivity.

PERFORMANCE CHARACTERISTICS

Specificity

Specificity of CONFIRM anti-S100 (Polyclonal) was determined by a study that showed appropriate staining of cells of neural and neuroendocrine origin. The following results were found in positive staining neoplasms: for melanoma, 16 out of 20 cases stained positive. The following results were found in negative staining neoplasms: for breast carcinoma, 0 out of 9 were positive; for carcinoid, 1 out of 10 was positive; for leiomyosarcoma, 0 out of 8 were positive; for Squamous cell carcinoma, 0 out of 10 were positive. A variety of formalin-fixed, paraffin-embedded normal tissues were stained with CONFIRM anti-S100 (Polyclonal) and the following tissues tested positive: adrenal, cervix, colon, endometrium, esophagus, mesothelium, ovary, peripheral nerve, pituitary, prostate, skin, small intestine, spleen and stomach. The following tissues tested negative: bone marrow, breast, heart, kidney, liver, lung, pancreas, salivary gland, muscle (skeletal), testis, thyroid and tonsil.

Sensitivity

Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens S100 detection by CONFIRM anti-S100 (Polyclonal) and may generate false negative results.

Repeatability

Intra-run reproducibility of staining with CONFIRM anti-S100 (Polyclonal) was determined by staining 10 slides containing the same tissue. Ten of ten slides stained positively. All slides stained with the same staining intensity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium and high antigen density in a single run.

Inter-run reproducibility of staining was determined by staining slides containing the same tissue on sixteen different instrument runs. Sixteen of sixteen slides stained positively. All slides stained with similar intensity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium and high antigen density on different days.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper barcode label. If the slide is labeled properly, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step may be included.
4. If specific antibody staining is too intense, the run should be repeated with the primary antibody incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
6. For corrective action, refer to the Step By Step Procedure section of the automated slide stainer Operator's Manual or contact your local support representative.

REFERENCES

1. Taylor RT and Cote RJ. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist, 2nd Edition. Philadelphia: W.B. Saunders Company; 1994.
2. True L. Atlas of Diagnostic Immunohistopathology. Philadelphia: J.B. Lippincott Company; 1990.
3. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980.
4. Kahn HJ, Marks A, Thom H, Baumal R. Role of Antibody to S100 Protein in Diagnostic Pathology. Amer J Clin Pathol. 1983;79(3): 341-347.

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