S100 Protein Cocktail

Concentrated and Prediluted Cocktail Antibody
901-089-092017

BIOCARE MEDICAL

Catalog Number:	**CM 089 A, B, C**	**PM 089 AA, H**	**IP 089 G10**	**OAI 089 T60**
Description:	0.1, 0.5, 1.0 ml, concentrated	6.0, 25 ml, prediluted	10 ml, prediluted	60 tests, prediluted
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Da Vinci Green	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
S100 Protein Cocktail [15E2E2 + 4C4.9] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of S100 proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
The S100 antibody cocktail recognizes proteins of 21-24 kDa, identified as A and B subunits of S100 protein. S100 belongs to the family of calcium binding proteins such as calmodulin and troponin C. S100A is composed of alpha and beta chains whereas S100B is composed of two beta chains. Antibody S100 stains melanocytes, schwannomas, peripheral neural tissue, astrocytes, benign and malignant melanomas, and their metastases (1-6). Studies have shown S100 protein is also expressed in the antigen presenting cells such as the Langerhans cells in skin and interdigitating reticulum cells in the paracortex of lymph nodes. Histiocytosis X can also be confirmed by S100 staining. According to studies, the S100 monoclonal cocktail is potentially more sensitive than other S100 single clone antibodies; and thus is an excellent pan-melanoma marker. S100 protein is highly soluble and may be eluted from frozen tissue during staining.

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human, mouse and rat
Clone: 15E2E2 + 4C4.9
Isotype: IgG2a + IgG2a
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: S100 protein
Cellular Localization: Cytoplasmic and nuclear
Positive Tissue Control: Melanoma
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH and manual use):
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: (recommended in most cases): Perform heat retrieval using Biocare's Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.

Protocol Recommendations (intelliPATH and manual use) Cont'd:
Optional: No Pretreatment (recommended for better morphology preservation in cutaneous melanomas)
Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT if no pretreatment used. Incubate for 15 minutes at RT if heat pretreatment used.
Probe: Incubate for 10 minutes at RT if no pretreatment used. Incubate for 5 minutes at RT if heat pretreatment used.
Polymer: Incubate for 10-20 minutes at RT if no pretreatment used. Incubate for 5 minutes at RT if heat pretreatment used.
Chromogen:
Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Biocare's Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
intelliPATH™ Automated Slide Stainer:
IP089 is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

Protocol Recommendations (ONCORE Automated Slide Staining System):
OAI089 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:
Protocol Name: S100
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR2, low pH; 70°C
Reagent Name, Time, Temp.: S100, 30 min., 25°C
Technical Note:
This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection, intelliPATH Universal HRP Detection Kit and ONCORE HRP Detection. Use TBS for washing steps.
Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011.

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Banerjee SS, *et al.* Malignant melanoma showing smooth muscle differentiation. J Clin Pathol. 1996 Nov;49(11):950-1.
2. Argenyi ZB, *et al.* S-100 protein-negative malignant melanoma: fact or fiction? A light- microscopic and immunohistochemical study. Am J Dermatopathol. 1994 Jun;16(3):233-40.
3. Fernando SS, Johnson S, Bate J. Immunohistochemical analysis of cutaneous malignant melanoma: comparison of S-100 protein, HMB45 monoclonal antibody and NKI/C3 monoclonal antibody. Pathology. 1994 Jan;26(1):16-9.
4. Tousignant J, *et al.* Immunohistochemical characteristics of malignant melanoma. A study of 40 cases and review of the literature. Arch Anat Cytol Pathol. 1990; 38(1-2):5-10.
5. Miettinen M, Franssila K. Immunohistochemical spectrum of malignant melanoma. The common presence of keratins. Lab Invest. 1989 Dec;61(6):623-8.
6. Fitzgibbons PL, *et al.* Primary mucosal malignant melanoma: an immunohistochemical study of 12 cases with comparison to cutaneous and metastatic melanomas. Hum Pathol. 1989 Mar;20(3):269-72.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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