

# S100 Protein [4C4.9] (M)

Concentrated and Prediluted Monoclonal Antibody  
901-3237-060123

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3237 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	API 3237 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3237 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 3237 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3237 G	6.0 mL	Ready-to-use	N/A
Q Series – For Leica BOND-III	ALI 3237 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

S100 Protein [4C4.9] (M) is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of S100 protein 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:

S100 recognizes proteins of 21-24 kDa, identified as the 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 an alpha and beta chain whereas S100B is composed of two beta chains. S100 protein is expressed in Schwannomas, ependymomas, astroglomas, and nearly all melanomas (benign and malignant) and their metastases (1-6). Studies have also shown S100 protein is expressed in Langerhans cell tumors and interdigitating dendritic cell tumor/sarcoma (IDCT) (7). Langerhans Cell Histiocytosis (also known as histiocytosis X, eosinophilic granuloma, or Langerhans cell granulomatosis) can also be confirmed by S100 staining (8).

## 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. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** 4C4.9

**Isotype:** IgG2a/kappa

**Protein Concentration:** 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 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM3237 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

## Protocol Recommendations (VALENT® Automated Slide Staining Platform) Cont'd:

### - DAB Chromogen Staining Option:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block:** Incubate for 10 minutes at RT with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

### - Red Chromogen Staining Option:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).

**Protein Block:** Incubate for 10 minutes with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Polymer:** Incubate for 45 min with Val Mouse AP Polymer.

**Chromogen:** Incubate for 15 min with Val Fast Red.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

**Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI3237 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** S100 - OR – S100 AP

**Protocol Template (Description):** Ms HRP Template 1 – OR – Ms AP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR1, high pH; 60°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** S100, 15 min., 25°C

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## **Protocol Recommendations (Ventana BenchMark ULTRA):**

AVI3237 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **OptiView**:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 16 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 4 minutes, 36°C

**Option 2 (V-Blocker BRI4001):** Incubate for 8 minutes (with appropriate Option # registered by user).

- Using **ultraView AP Red**:

**Template/Detection:** ultraView Red

**Pretreatment Protocol:** CC1 Mild

**Primary Antibody:** 8 minutes, 36°C

**ultraBlock (V-Blocker BRI4001):** Incubate for 8 minutes (with appropriate Option # registered by user)

## **Protocol Recommendations (Q Series – For Leica BOND-III):**

ALI3237 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- **DAB Chromogen Staining Option:**

**Protocol Name:** IHC Protocol F

**Detection:** Bond Polymer Refine

**HIER:** 20 min with ER1

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

- **Red Chromogen Staining Option:**

**Protocol Name:** IHC Protocol J

**Detection:** Bond Polymer Refine Red

**HIER:** 20 min with ER1

**Marker (Primary Antibody):** 15 min

**Post Primary AP:** 20 min

**Polymer AP:** 30 min

**Mixed Red Refine:** 10 min + 5 min

**Hematoxylin:** 5 min

## **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.

## **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<sub>3</sub>) 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) (9)

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

## **Precautions Cont'd:**

or specimens come into contact with sensitive areas, wash with copious amounts of water. (10)

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:**

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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. Pileri SA, *et al.* Tumours of histiocytes and accessory dendritic cells: an immunohistochemical approach to classification from the International Lymphoma Study Group based on 61 cases. *Histopathology.* 2002 Jul;41(1):1-29.
8. Redd L, *et al.* Langerhans Cell Histiocytosis Shows Distinct Cytoplasmic Expression of Major Histocompatibility Class II Antigens. *J Hematop.* 2016 Sep;9(3):107-112.
9. 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."
10. 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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