

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects recombinant mouse S100A4 protein in Direct ELISA
Source	Monoclonal Rat IgG _{2B} Clone # 1092141
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived mouse S100A4 Ala2-Lys101 Accession # P07091
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

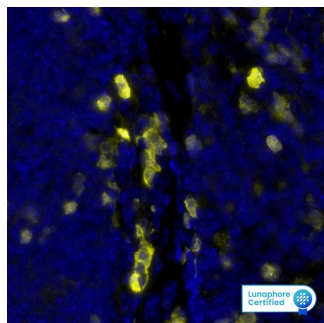
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	NIH-3T3 mouse embryonic fibroblast cell line
Multiplex Immunofluorescence	0.5 µg/mL	Immersion fixed paraffin-embedded sections of mouse spleen
Immunohistochemistry	3-25 µg/mL	Immersion fixed paraffin-embedded sections of mouse spleen
Simple Western	20 µg/mL	NIH-3T3 mouse embryonic fibroblast cell line

DATA

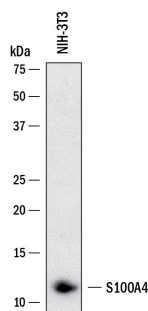
Multiplex Immunofluorescence



S100A4 in Mouse Spleen via seqIF™ staining on COMET™

S100A4 was detected in immersion fixed paraffin-embedded sections of mouse Spleen using Rat Anti-Mouse S100A4, Monoclonal Antibody (Catalog #MAB11673) at 0.5µg/mL at 32° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Eprelia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 555 Goat anti-Rat IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555RT) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm and nucleus. Protocol available in [COMET™ Panel Builder](#).

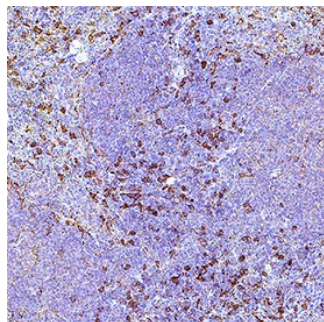
Western Blot



Detection of Mouse S100A4 by Western Blot.

Western Blot shows lysates of NIH-3T3 mouse embryonic fibroblast cell line. PVDF membrane was probed with 1 µg/ml of Rat Anti-Mouse S100A4 Monoclonal Antibody (Catalog # MAB11673) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for S100A4 at approximately 11 kDa (as indicated). This experiment was conducted under reducing conditions and using [Western Blot Buffer Group 1](#).

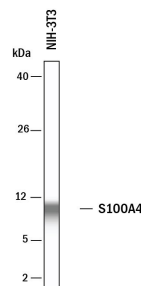
Immunohistochemistry



Detection of S100A4 in Mouse Spleen.

S100A4 was detected in immersion fixed paraffin-embedded sections of mouse spleen using Rat Anti-Mouse S100A4 Monoclonal Antibody (Catalog # MAB11673) at 5 µg/ml overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using the HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005) and counterstained with hematoxylin (blue). Specific staining was localized to the nucleus and cytoplasm. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Simple Western



Detection of Mouse S100A4 by Simple Western™.

Simple Western shows lysates of NIH-3T3 mouse embryonic fibroblast cell line, loaded at 0.5 mg/ml. A specific band was detected for S100A4 at approximately 10 kDa (as indicated) using 20 µg/mL of Rat Anti-Mouse S100A4 Monoclonal Antibody (Catalog # MAB11673). This experiment was conducted under reducing conditions and using the 2-40kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

S100A4 (also named Metastasin, Mts1 and Calvasculin) is an 11 kDa member of the S100 (soluble in 100% saturated ammonium sulfate) family of proteins (1-5). S100 family members belong to the EF-hand superfamily of Ca⁺⁺-binding proteins. These participate in both calcium-dependent and calcium-independent protein-protein interactions. The hallmark of this superfamily is the EF-hand motif that consists of a Ca⁺⁺-binding site flanked by two α -helices (helix E and helix F) that were originally identified in a right-handed model of carp muscle calcium-binding protein (6). Mouse S100A4 is 101 amino acids (aa) in length (1, 2). It contains two EF hand domains (aa 12-47 and aa 50-85). The first domain has a 14 aa cation-binding motif and binds Ca⁺⁺ with low affinity. The second Ca⁺⁺-binding motif is 12 aa in length and binds Ca⁺⁺ with high affinity. S100A4 has no classical signal sequence but is secreted from cells (3, 7). Mouse S100A4 shares 93%, 96% and 89% aa identity with human, rat and canine S100A4, respectively. S100A4 exists as dimer (8, 9, 10). Extracellular S100A4 is reported to induce MMP production, activate MMPs, promote neurite outgrowth and stimulate cardiomyocyte proliferation (4, 10, 11, 12, 13). Within the cell, dimers are likely the functional unit. Here, they are constitutive homo- or heterodimers (with S100A1) that interact with Ca⁺⁺, undergo a conformational change, and subsequently bind to cytoplasmic targets. Known targets include p53, Myosin heavy chain II, F-actin and Liprin β 1 (4, 14). In general, it can be said that S100A4 blocks target phosphorylation and multimerization (4, 7, 14). S100A4 activity has been associated with cell transformation. It seems likely this is either coincidental, or a consequence, rather than a cause of transformation (3).

References:

1. Jackson-Grusby, L.L. *et al.* (1987) *Nucleic Acids Res.* **15**:6677.
2. Goto, K. *et al.* (1988) *J. Biochem.* **103**:48.
3. Garrett, S.C. *et al.* (2006) *J. Biol. Chem.* **281**:677.
4. Santamaria-Kisiel, L. *et al.* (2006) *Biochem. J.* **396**:201.
5. Donato, R. (2001) *Int. J. Biochem. Mol. Biol.* **33**:637.
6. Kretsinger, R.H. and C.E. Nockolds (1973) *J. Biol. Chem.* **248**:3313.
7. Helfman, D.M. *et al.* (2005) *Br. J. Cancer* **92**:1955.
8. Burkitt, W.I. *et al.* (2003) *Biochem. Soc. Trans.* **31**:985.
9. Vallaly, K.M. *et al.* (2002) *Biochemistry* **41**:12670.
10. Novitskaya, V. *et al.* (2000) *J. Biol. Chem.* **275**:41278.
11. Stary, M. *et al.* (2006) *Biochem. Biophys. Res. Commun.* **343**:555.
12. Semov, A. *et al.* (2005) *J. Biol. Chem.* **280**:20833.
13. Saleem, M. *et al.* (2006) *Proc. Natl. Acad. Sci. USA* **103**:14825.
14. Kriajevska, M. *et al.* (2002) *J. Biol. Chem.* **277**:5229.