Human S100A9 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF5578

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human S100A9 in direct ELISAs and Western blots. In direct ELISAs, less than 5% cross-reactivity with recombinant mouse S100A9 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human S100A9 The2-Pro114 Accession # P06702
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

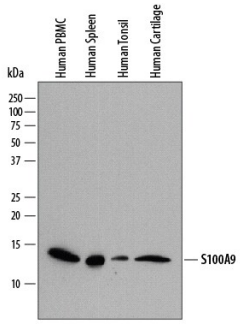
APPLICATIONS

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	0.2 µg/mL	See Below
Immunocytochemistry	1-25 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Knockout Validated	S100A9 is specifically detected in MDA-MB-468 human breast cancer parental cell line but is not detectable in S100A9 knockout MDA-MB-468 cell line.	

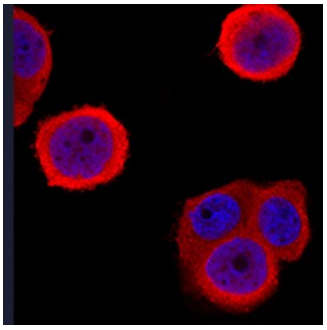
DATA

Western Blot



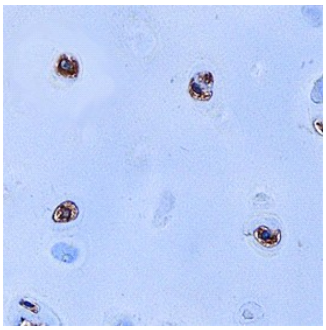
Detection of Human S100A9 by Western Blot. Western blot shows lysates of human peripheral blood mononuclear cells (PBMC), human spleen tissue, human tonsil tissue, and human cartilage tissue. PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human S100A9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5578) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for S100A9 at approximately 14 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



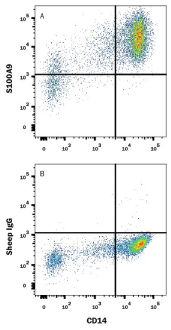
S100A9 in MDA-MB-468 Human Cell Line. S100A9 was detected in immersion fixed MDA-MB-468 human breast cancer cell line using Sheep Anti-Human S100A9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5578) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



S100A9 in Human Cartilage. S100A9 was detected in immersion fixed paraffin-embedded sections of human cartilage using Sheep Anti-Human S100A9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5578) at 1 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm of chondrocytes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Intracellular Staining by Flow Cytometry



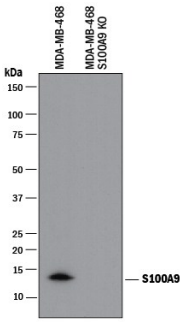
Detection of S100A9 in Human PBMCs by Flow Cytometry. Human peripheral blood monocytes (PBMC) were stained with Mouse Anti-Human CD14 PE-conjugated Monoclonal Antibody (Catalog # FAB3832P) and either (A) Sheep Anti-Human S100A9 Polyclonal Antibody (Catalog # AF5578) or (B) Sheep IgG Isotype Control (Catalog # 5-001-A) followed by anti-Sheep IgG APC-conjugated Secondary Antibody (Catalog # F0127). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer (Catalog # FC005). View our protocol for Staining Membrane-associated Proteins.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Human S100A9 Antibody

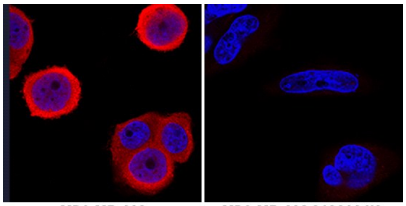
Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF5578

Knockout Validated



Western Blot Shows Human S100A9 Specificity by Using Knockout Cell Line. Western blot shows lysates of MDA-MB-468 human breast cancer parental cell line and S100A9 knock out MDA-MB-468 cell line (KO). PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human S100A9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5578) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for S100A9 at approximately 14 kDa (as indicated) in the parental MDA-MB-468 cell line, but is not detectable in knockout MDA-MB-468 cell line. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



S100A9 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. S100A9 was detected in immersion fixed MDA-MB-468 human breast cancer cell line but is not detected in S100A9 knockout (KO) MDA-MB-468 cell line using Sheep Anti-Human S100A9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5578) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 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

Human S100A9 (also MRP-14, Calgranulin-B, and p14) is a 14 kDa member of the S100 family of EF-hand calcium-binding proteins. It is 114 amino acids (aa) in length and contains short sequential modules. There is an N-terminal helical region, followed by a calcium-binding EF-hand domain, two more helical regions, a second EF-hand domain, and three additional helical regions. S100A9 will noncovalently heterodimerize with S100A8. In the presence of calcium, this heterodimer will form a heterotetramer. S100A9 is expressed in granulocytes, monocytes, and macrophages during acute and chronic inflammation. Human S100A9 shares 62% and 57% aa identity with rat and mouse S100A9, respectively.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449