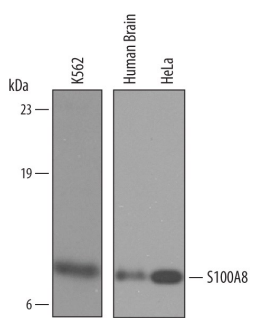
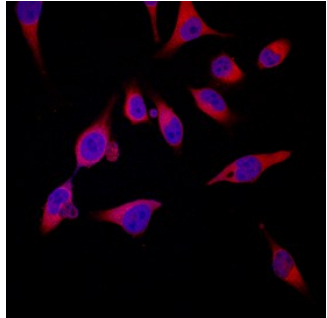
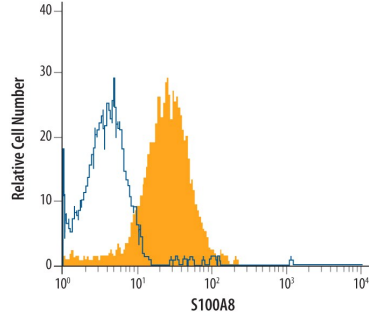
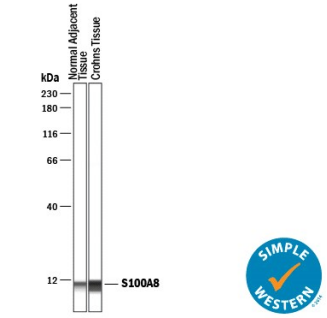


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human S100A8 in direct ELISAs and Western blots. In these formats, less than 1% cross-reactivity with recombinant mouse S100A8, recombinant human (rh) S100A1, and rhS100P is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human S100A8 Met1-Glu93 Accession # P05109
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. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.		
	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
Simple Western	10 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

DATA	
<p>Western Blot</p>  <p>Detection of Human S100A8 by Western Blot. Western blot shows lysates of human brain tissue, K562 human chronic myelogenous leukemia cell line, and HeLa human cervical epithelial carcinoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human S100A8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4570) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for S100A8 at approximately 10 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.</p>	<p>Immunocytochemistry</p>  <p>S100A8 in HeLa Human Cell Line. S100A8 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human S100A8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4570) at 10 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.</p>

<p>Intracellular Staining by Flow Cytometry</p>  <p>Detection of S100A8 in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Sheep Anti-Human S100A8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4570), filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by NorthernLights™ 637-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL011). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.</p>	<p>Simple Western</p>  <p>Detection of Human S100A8 by Simple Western™. Simple Western lane view shows lysates of normal adjacent tissue and Crohns tissue, loaded at 0.2 mg/mL. A specific band was detected for S100A8 at approximately 10 kDa (as indicated) using 20 µg/mL of Sheep Anti-Human S100A8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4570) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.</p>
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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. <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

S100A8 (also known as MRP8 and calgranulin A) is a 10 kDa member of the S100 family, EF-hand superfamily of Ca-binding proteins. It is produced by neutrophils and monocytes, and forms Ca-dependent heterodimer/heterotetramer complexes (termed calprotectin) with S100A9. It functions both intracellularly and extracellularly, where it binds to RAGE and CD36. Human S100A8 is 93 amino acids (aa) in length. It contains two EF-hand motifs (aa 12-47 and 46-81) and one high-affinity Ca-binding site (aa 59-70). There may be one splice form that shows a 15 aa substitution for the C-terminal 14 amino acids. Although mouse S100A8 is cleaved by MMP-2 after Asn21, it is unclear if human S100A8 is susceptible. Full-length human S100A8 is 57% and 74% aa identical to mouse and canine S100A8, respectively.