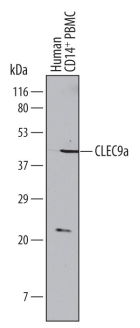
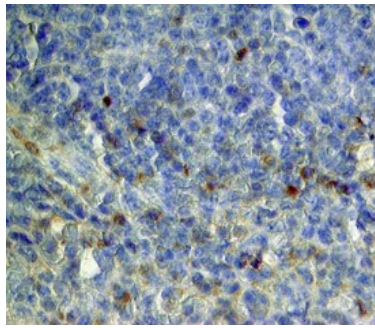


DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human CLEC9a in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant mouse CLEC9a is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CLEC9a Lys57-Val241 Accession # Q6UXN8
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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
Immunohistochemistry	5-15 µg/mL	See Below
Binding Inhibition	The binding of Recombinant Human CLEC9a Fc Chimera (Catalog # 6049-CL, 100 ng/mL) to a ligand expressed in Fas Ligand-treated A20 mouse B cell lymphoma cell line was maximally inhibited (>80%) by 10 µg/mL of the antibody, as detected by flow cytometry.	

DATA	
<p>Western Blot</p>  <p>Detection of Human CLEC9a by Western Blot. Western blot shows lysates of human CD14⁺ peripheral blood mononuclear cells (PBMC). PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human CLEC9a Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6049) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for CLEC9a at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.</p>	<p>Immunohistochemistry</p>  <p>CLEC9a in Human Spleen. CLEC9a was detected in immersion fixed paraffin-embedded sections of human spleen using Sheep Anti-Human CLEC9a Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6049) at 15 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. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>

PREPARATION AND STORAGE	
Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

CLEC9a (C-type lectin domain family 9 member A), also known as DNGR-1, is a type II transmembrane glycoprotein member of the C-type lectin superfamily (1, 2). Mature human CLEC9a consists of a 35 amino acid (aa) cytoplasmic domain with an ITAM-like motif, a 21 aa transmembrane segment, and a 185 extracellular domain (ECD) that contains a stalk region and one C-type lectin domain (CTLD) (3-5). Within the ECD, human CLEC9a shares 57% aa sequence identity with mouse and rat CLEC9a. Although the CTLD of CLEC9a structurally resembles that of other C-type lectins, it lacks the conserved residues that typically mediate calcium and carbohydrate binding. CLEC9a is expressed as a disulfide-linked homodimer of approximately 45-50 kDa N-glycosylated subunits (3, 5). Human CLEC9a expression is restricted to a subpopulation of BDCA-3⁺ conventional dendritic cells (cDC) and CD16⁻ monocytes (3-5). BDCA-3⁺ cDC are analogous to mouse CD8⁺ cDC which are specialized in antigenic cross-presentation in complex with MHC class I molecules (6). In mouse, CLEC9a is additionally expressed on plasmacytoid dendritic cells (4, 5). CLEC9a ligation enhances antigen uptake and processing, leading to presentation on MHC class I and cytotoxic T cell (CTL) priming (3-5). In mouse, CLEC9a recognizes normally intracellular determinant(s) of necrotic cells and mediates their uptake by the dendritic cell (7). The subsequent antigenic cross-presentation to CTL is important for clearing necrotic cellular debris (7). CLEC9a signaling triggers activation of the tyrosine kinase Syk (3, 7).

References:

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