Human CLEC9a Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6049

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 as a 0.2 µm filtered solution in PBS.

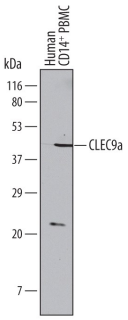
APPLICATIONS

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	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.	

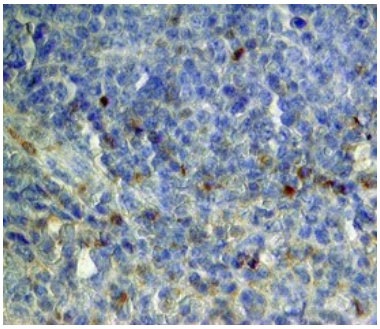
DATA

Western Blot



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.

Immunohistochemistry



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.

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.** ● 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.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

Human CLEC9a Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6049

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 analagous 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:

1. Huysamen, C. and G.D. Brown (2009) FEMS Microbiol. Lett. **290**:121.
2. Geijtenbeek, T.B.H. *et al.* (2004) Annu. Rev. Immunol. **22**:33.
3. Huysamen, C. *et al.* (2008) J. Biol. Chem. **283**:16693.
4. Caminschi, I. *et al.* (2008) Blood **112**:3264.
5. Sancho, D. *et al.* (2008) J. Clin. Invest. **118**:2098.
6. Dudziak, D. *et al.* (2007) Science **315**:107.
7. Sancho, D. *et al.* (2009) Nature **458**:899.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956