

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human 2B4/CD244/SLAMF4 in ELISAs and Western blots. In sandwich immunoassays, less than 0.2% cross-reactivity with recombinant mouse 2B4, recombinant human (rh) SLAM, rhCD48, and rhCD84 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human 2B4/CD244/SLAMF4 Cys22-Arg221 Accession # NP_057466
Endotoxin Level	<0.01 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.

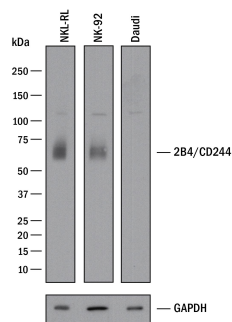
APPLICATIONS

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

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Human 2B4/CD244/SLAMF4 Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 µg/mL	Human 2B4/CD244/SLAMF4 Antibody (Catalog # AF1039)
ELISA Detection Standard	0.1-0.4 µg/mL	Human 2B4/CD244/SLAMF4 Biotinylated Antibody (Catalog # BAF1039) Recombinant Human 2B4/CD244/SLAMF4 Fc Chimera (Catalog # 1039-2B)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Agonist Activity	Measured by its ability to induce IFN-γ secretion by NK-92 human natural killer lymphoma cells. The ED ₅₀ for this effect is typically 4-12 µg/mL.	

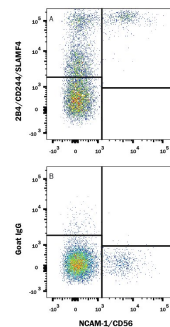
DATA

Western Blot



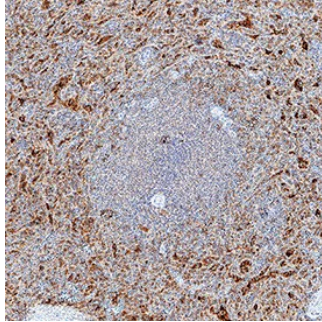
Detection of Human 2B4/CD244/SLAMF4 by Western Blot. Western blot shows lysates of NK-RL human natural killer lymphocytic leukemia cell line, NK-92 human natural killer lymphoma cell line, and Daudi human Burkitt's lymphoma cell line (negative control). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human 2B4/CD244/SLAMF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1039) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for 2B4/CD244/SLAMF4 at approximately 65 kDa (as indicated). GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of 2B4/CD244/SLAMF4 in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with Mouse Anti-Human NCAM-1/CD56 APC-conjugated Monoclonal Antibody (Catalog # FAB2408A) and either (A) Goat Anti-Human 2B4/CD244/SLAMF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1039) or (B) Normal Goat IgG Control (Catalog # AB-108-C) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107).

Immunohistochemistry



2B4/CD244/SLAMF4 in Human Spleen.
2B4/CD244/SLAMF4 was detected in immersion fixed paraffin-embedded sections of human spleen using Goat Anti-Human 2B4/CD244/SLAMF4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1039) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to splenocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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	<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

2B4, also known as CD244 and SLAMF4, is a 66 kDa type I transmembrane glycoprotein in the SLAM subgroup of the CD2 protein family. SLAM family proteins have an extracellular domain (ECD) with two or four Ig-like domains and at least two cytoplasmic immunoreceptor tyrosine-based switch motifs (ITSMs). 2B4 interacts with CD48, while other SLAM family proteins interact homophilically (1-4). Mature human 2B4 consists of a 208 amino acid (aa) ECD with two Ig-like domains, a 21 aa transmembrane segment, and a 120 aa cytoplasmic domain with four ITSMs (5, 6). Three additional splice variants of human 2B4 have deletions of the short region between the Ig-like domains, the second Ig-like domain, or a portion of the cytoplasmic tail. Within the ECD, human 2B4 shares 46% and 40% aa sequence identity with mouse and rat 2B4, respectively. The ECD of human 2B4 shares 17%-24% aa sequence identity with comparable regions of human CD2 family members BLAME, CD2F-10, CD84, CD229, CRACC, NTB-A, and SLAM. 2B4 is expressed on all NK cells, $\gamma\delta$ T cells, monocytes, some CD4⁺ and CD8⁺ T cells, and some dendritic cells (7). CD48 mediates 2B4⁺ cell interactions with nearly all hematopoietic cell types, including cells of the same type (8-10). 2B4/CD48 signaling cooperates with other receptor systems to either promote or inhibit NK and CD8⁺ T cell activation (7-13). The inhibitory activities are distinct from those of MHC I restricted inhibitory NK cell receptors (12, 13). Ligation of 2B4 with antibodies or CD48 constructs can either directly trigger inhibitory signaling or disrupt an inhibitory interaction, leading to cellular activation (9, 12). The inhibitory effect is associated with the long form of 2B4, while the activation is associated with the short form (9, 14). 2B4 can also induce signaling through CD48 (10, 15).

References:

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