Human CD99 Antibody

Monoclonal Mouse IgG_1 Clone # 1021527

Catalog Number: MAB3968

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD99 in direct ELISAs.
Source	Monoclonal Mouse IgG_1 Clone # 1021527
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived human CD99 Asp23-Asp122 Accession # P14209
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.

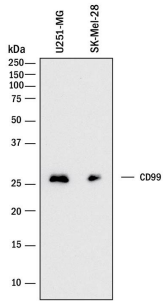
APPLICATIONS

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	2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

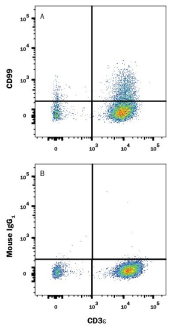
DATA

Western Blot



Detection of Human CD99 by Western Blot. Western blot shows lysates of U251-MG human malignant glioblastoma cell line and SK-Mel-28 human malignant melanoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human CD99 Monoclonal Antibody (Catalog # MAB3968) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for CD99 at approximately 30 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of CD99 in Human PBMC Lymphocytes by Flow Cytometry. Human peripheral blood mononuclear cell (PBMC) lymphocytes were stained with (A) Mouse Anti-Human CD99 Monoclonal Antibody (Catalog # MAB3968) or (B) Mouse IgG1 Isotype Control (Catalog # MAB002) followed by anti-Mouse IgG PE-conjugated secondary antibody (Catalog # F0102B) and Mouse Anti-Human CD3 APC-conjugated Monoclonal Antibody (Catalog # FAB100A). View our protocol for Staining Membrane-associated Proteins.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
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BACKGROUND

CD99 (also named MIC2, E2 and thymic leukemia antigen) is the founding member of the CD99 family of molecules. The CD99 family contains four members; CD99, CD99L2, XG and the pseudogene CD99L1 (1, 2, 3). Native human CD99 is 32 kDa in size and exists as a type I transmembrane glycoprotein. This is referred to as the long, or type I isoform. It is synthesized as a 185 amino acid (aa) precursor that contains a 22 aa signal sequence, a 100 aa extracellular domain (ECD), a 25 aa transmembrane segment, and a 38 aa cytoplasmic region (4). The ECD contains no identifiable motifs, N-linked glycosylation sites, or cysteine residues; it does possess sites for O-linked glycosylation. The cytoplasmic region, albeit short, does have signal transduction capability (5). There are apparently multiple isoforms for human CD99. One shows a 16 aa deletion in the ECD (aa 34 - 49), a second shows a 38 aa deletion in the cytoplasmic region (aa 122 - 159), and a third exhibits a three aa truncation at the C-terminus (6, 7, 8). The best studied isoform shows an Asp-Gly substitution for the C-terminal 27 amino acids. This is referred to as the 28 kDa type II isoform (9). The type I and II isoforms have distinctive signal transduction pathways (FAK-src for type I; PI3K plus src-ERK1/2 for type II), and mediate clearly different biological outcomes (5, 9, 10). The two numbered isoforms may or may not coexist on the same cells. Peripheral T cells have only the long isoform, while double-positive thymocytes express both isotypes. What is unclear is the monomeric vs. dimeric status of CD99. In mouse, CD99 reportedly forms disulfide-linked homodimers (11). In human, however, CD99 is reportedly monomeric if only a type I isoform, and a covalent heterodimer if coexpressing type I and II isoforms (12, 13). Cells known to express CD99 include fibroblasts, neutrophils, T cells, double-positive thymocytes, CD34+ stem cells, monocytes and endothelial cells (2, 12, 14, 15). Homophilic interaction between CD99 on the neutrophil and CD99 on the endothelial cell regulates the transendothelial migration of neutrophils during inflammation (16). Human CD99 is only 48% aa identical to mouse CD99 (17).

References:

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