

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD97 in direct ELISAs and Western blots. In direct ELISAs and Western blots, no cross-reactivity with recombinant mouse CD97 is observed.
Source	Monoclonal Mouse IgG _{2A} Clone # 380903
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CD97 Gln21-Gln398 Accession # NP_001775.2
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	Recombinant Human CD97 (Catalog # 2529-CD) under non-reducing conditions only
Flow Cytometry	2.5 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.	
Neutralization	Measured by its ability to neutralize CD97-mediated adhesion of human red blood cells. Hamann, J. <i>et al.</i> (1996) <i>J. Exp. Med.</i> 184 :1185. The Neutralization Dose (ND ₅₀) is typically 0.5-2.5 µg/mL in the presence of 4 µg/mL Recombinant Human CD97.	

DATA

Flow Cytometry

Detection of CD97 in Human Whole Blood by Flow Cytometry. Human whole blood was stained with Mouse Anti-Human CD97 Monoclonal Antibody (Catalog # MAB2529, filled histogram) or isotype control antibody (Catalog # MAB0031, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab')₂ Secondary Antibody (Catalog # F0102B).

Neutralization

Cell Adhesion Mediated by CD97 and Neutralization by Human CD97 Antibody. Recombinant Human CD97 (Catalog # 2529-CD), immobilized onto a microplate, supports the adhesion of human red blood cells in a dose-dependent manner (orange line). Adhesion elicited by Recombinant Human CD97 (4 µg/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human CD97 Monoclonal Antibody (Catalog # MAB2529). The ND₅₀ is typically 0.5-2.5 µg/mL.

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. <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

CD97 is a 95-100 kDa member of a protein group known as the LNB-TM7 protein family that evolved from genes of the secretin receptor superfamily (1-3). Molecules in this family are unique hybrid structures consisting of EGF-like modules coupled to class B G-protein 7-transmembrane (TM) domains by a glycosylated (mucin) stalk. Human CD97 is synthesized as an 835 amino acid (aa) precursor that contains a 20 aa signal sequence, a 532 aa extracellular domain (ECD), a 238 aa "transmembrane" region that includes seven TM segments, and a 45 aa cytoplasmic tail (4). Within the 532 aa ECD, the first 236 aa contains five EGF-like domains, the C-terminal four of which bind calcium, and a juxtamembrane 296 aa RGD-containing mucin stalk (4, 5). The stalk is both glycosylated and proteolytically cleaved (at aa 530) to create a noncovalently linked 65-70 kDa glycosylated extracellular α -subunit and a 28 kDa 7-TM membrane-bound β -subunit (4). There are two known alternate splice forms in human. Isoform # 1 contains four EGF-like domains (domain # 1, 2, 3 and 5), while isoform # 2 contains three EGF-like domains (domain # 1, 2 and 5) (1, 4, 6). The ECD in isoform 1 is 60 kDa in size, while the ECD in isoform 2 is 55 kDa in size (native molecular weight). The five EGF-like domain region in human is 55% aa identical to that in mouse. Cells known to express CD97 include monocytes, macrophages, T cells, select B cells, dendritic cells and, potentially, vascular and visceral smooth muscle cells (1, 7). There are at least two ligands for CD97. One is chondroitin sulfate that binds only to the full-length (five domain) form of CD97. Binding is dependent on the presence of EGF-like domain # 4 (3). The second ligand for CD97 is CD55, a GPI-linked cell surface molecule with short consensus repeats that regulates complement activation on cell surfaces (1, 5, 7). CD97 EGF-like domains # 1 and 2 bind CD55 while domain # 5 stabilizes the CD97 molecule. The shortest CD97 isoform shows the strongest binding to CD55 (7, 8).

References:

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5. Lin, H-H. *et al.* (2001) *J. Biol. Chem.* **276**:24160.
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