Human LAMP-2/CD107b Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF6228

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human LAMP2/CD107b in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human LAMP1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human LAMP2/CD107b Leu29-Phe375 Accession # P13473
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.

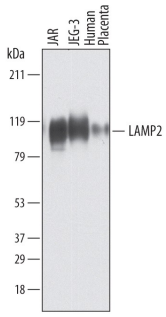
APPLICATIONS

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	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by 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.	
Knockout Validated	LAMP-2/CD107b is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in LAMP-2/CD107b knockout HeLa cell line.	

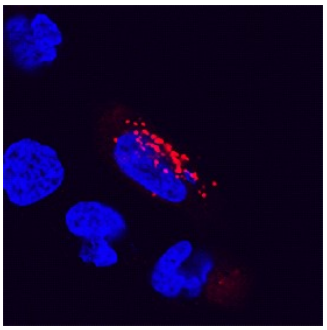
DATA

Western Blot



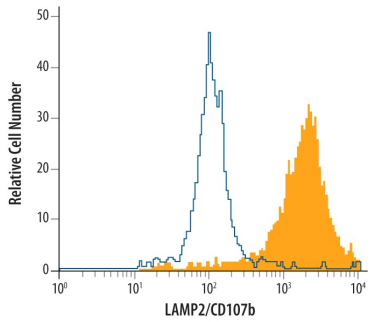
Detection of Human LAMP2/CD107b by Western Blot. Western blot shows lysates of JAR human choriocarcinoma cell line, JEG-3 human epithelial choriocarcinoma cell line, and human placenta tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human LAMP2/CD107b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6228) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). Specific bands were detected for LAMP2/CD107b at approximately 100-120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



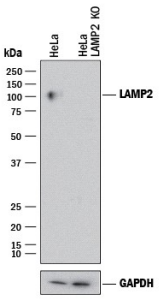
LAMP2/CD107b in HeLa Human Cell Line. LAMP2/CD107b was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human LAMP2/CD107b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6228) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to lysosomes. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of LAMP-2A in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Goat Anti-Human LAMP2/CD107b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6228, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Knockout Validated



Western Blot Shows Human LAMP-2/CD107b Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and LAMP-2/CD107b knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human LAMP-2/CD107b Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6228) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for LAMP-2/CD107b at approximately 100 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Human LAMP-2/CD107b Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF6228

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.

BACKGROUND

Lysosomal associated membrane protein 2 (LAMP2), also known as CD107b and LGP110, is an approximately 110 kDa transmembrane glycoprotein that is a major component of lysosomal membranes (1). Mature human LAMP2 consists of a 347 amino acid (aa) intralumenal domain, a 24 aa transmembrane segment, and a 35 aa cytoplasmic tail (2). Its lumenal domain is organized into two heavily N-glycosylated regions separated by a Ser/Pro-rich linker that carries a minor amount of O-linked glycosylation (2, 3). Alternate splicing generates a human LAMP2 isoform (LAMP2B) with a substituted juxtamembrane lumenal region, transmembrane segment, and cytoplasmic tail (4). Within the lumenal domain, human LAMP2 shares approximately 64% aa sequence identity with mouse and rat LAMP2. LAMP2 itself is subject to lysosomal degradation following cleavage of its lumenal domain (5). It mediates the lysosomal uptake of the chaperone HSC73 in complex with cargo proteins and is required for the lysosomal destruction of autophagic vacuoles (6, 7). In cytotoxic T cells and mast cells, LAMP2 is expressed in the membranes of intracellular granules that contain effector molecules such as perforin, granzymes, eicosanoids, and histamine (8-10). Up-regulated LAMP2 at the plasma membrane serves as an indicator of cell activation of $CD8^+$ T cells, mast cells, monocytes, and platelets (9-12). LAMP2 is a native ligand for lectins Galectin-1 and Galectin-3 (13-15).

References:

1. Eskelinen, E.-L. *et al.* (2003) Trends Cell Biol. **13**:137.
2. Fukuda, M. *et al.* (1988) J. Biol. Chem. **263**:18920.
3. Carlsson, S.R. *et al.* (1988) J. Biol. Chem. **263**:18911.
4. Konecki, D.S. *et al.* (1995) Biochem. Biophys. Res. Commun. **215**:757.
5. Cuervo, A.M. and J.F. Dice (2000) Traffic **1**:570.
6. Cuervo, A.M. and J.F. Dice (1996) Science **273**:501.
7. Tanaka, Y. *et al.* (1990) Nature **406**:902.
8. Peters, P.J. *et al.* (1991) J. Exp. Med. **173**:1099.
9. Betts, M.R. *et al.* (2003) J. Immunol. Meth. **281**:65.
10. Grutzkau, A. *et al.* (2004) Cytometry **61**:62.
11. Kannan, K. *et al.* (1996) Cell. Immunol. **171**:10.
12. Silverstein, R.L. and M. Febbraio (1992) Blood **80**:1470.
13. Skrincosky, D.M. *et al.* (1993) Cancer Res. **53**:2667.
14. Inohara, H. and Raz, A. (1994) Biochem. Biophys. Res. Commun. **201**:1366.
15. Ohannesian D.W. *et al.* (1994) Cancer Res. **54**:5992.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449