COSMO BIO CO., LTD.
Inspiration for Life Science

MONOCLONAL ANTIBODY

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Catalog No.LKG-M004, LKG-M005

Anti-Human LAT1-CD98 Rat IgG Monoclonal

BACKGROUND

LAT1 (L-type amino-acid transporter 1) is an amino-acid transporter which has 12 transmembrane domains. It functions as a disulfide-linked heterodimer with CD98 heavy chain (CD98hc). This heterodimeric complex of CD98hc and LAT1 (CD98C5) transports large and neutral amino acids (Leucine, Isoleucine, Valine, Phenylalanine, Tyrosine, Tryptophan, Methionine and Histidine) through the plasma membrane[1,2]. CD98C5 complex is known to be frequently and specifically overexpressed in a variety of cancer patients, and is a novel candidate as a molecular target of human cancer therapy[3-6]. It is difficult to make antibodies recognizing extracellular domain of LAT1 because it is less than 30 aa. We successfully made the high affinity antibodies against native LAT1 extracellular membrane domain of CD98C5 complex.

Product type	Primary Antibodies
Immunogen	Human LAT1-CD98 (CD98C5) complex expressing cancer cell
Raised in	Rat
Myeloma	P3 × 63Ag8.653
Clone number	RH01
Isotype	IgG2a
Source	Ascites
Purification	Affinity purified by Protein G
Buffer	Phosphate buffered saline (PBS)* *NOTE: PBS doesn't contain preservative. Preservative is added based on the research purpose.
Concentration	1mg / mL
Volume	Catalog No. LKG-M004: 50 uL (50 ug), Catalog No. LKG-M005: 100 uL (100 ug)
Label	Unlabeled
Specificity	Human LAT1 extracellular domain of CD98C5 complex.
Cross reactivity	Other species are not tested.
Storage	Store at 4 °C

Application notes **Recommended dilutions**	Flow cytometry; 5-20µg/mL Immunofluorescence; 5µg/mL - Other applications have not been tested. - Optimal dilutions/concentrations should be determined by the end user. - Detailed procedure is provided in the following PROTOCOLS.

References

1) Zhao Y., *et al*., Intractable Rare Dis Res. 2015 Nov;4(4):165-9. PMID: 26668776
2) Kanai Y., *et al*., J Biol Chem. 1998 Sep 11;273(37):23629-32. PMID: 9726963
3) Ohkawa M., *et al*., Biochem Biophys Res Commun. 2011 Mar 25;406(4):649-55. PMID: 21371427
4) Nawashiro H., *et al*., Brain Tumor Pathol. 2005;22(2):89-91. PMID: 18095110
5) Ichinoe M., *et al*., Pathol Int. 2011 May;61(5):281-9. PMID: 21501294
6) Oda K., *et al*., Cancer Sci. 2010 Jan;101(1):173-9. PMID: 19900191

Application data

Flow cytometry (based on protocol in page 3)

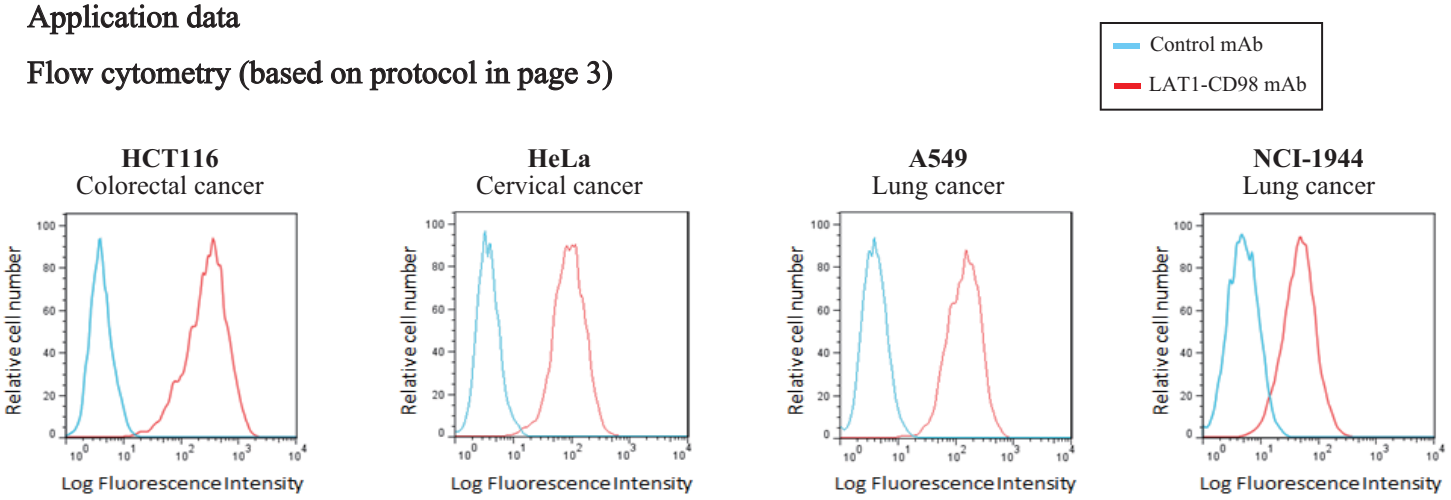

Fig 1. Flow cytometry analysis of LAT1-CD98 in Human cancer cell lines with anti-LAT1-CD98 (20µg/mL) antibody and PE-labeled anti Rat IgG antibody.

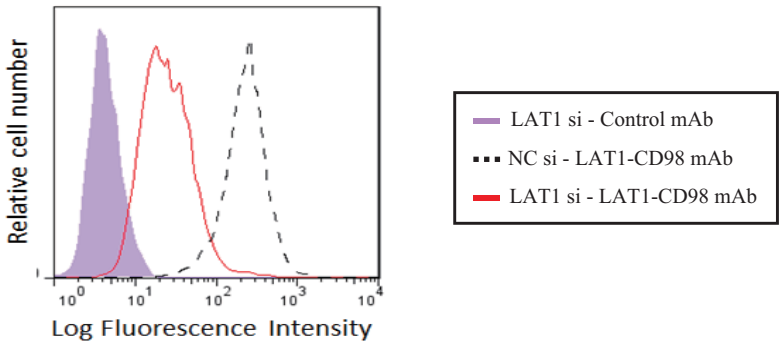

Fig 2. Flow cytometry analysis of LAT1-CD98 in HCT116 cells with anti-LAT1-CD98 (5µg/mL) antibody and PE-labeled anti Rat IgG antibody.
- LAT1-si: LAT1 knockdown samples were reduced their signal.
- NC-si: non-targeting siRNA treated sample

Immunofluorescence (based on protocol in page 3)

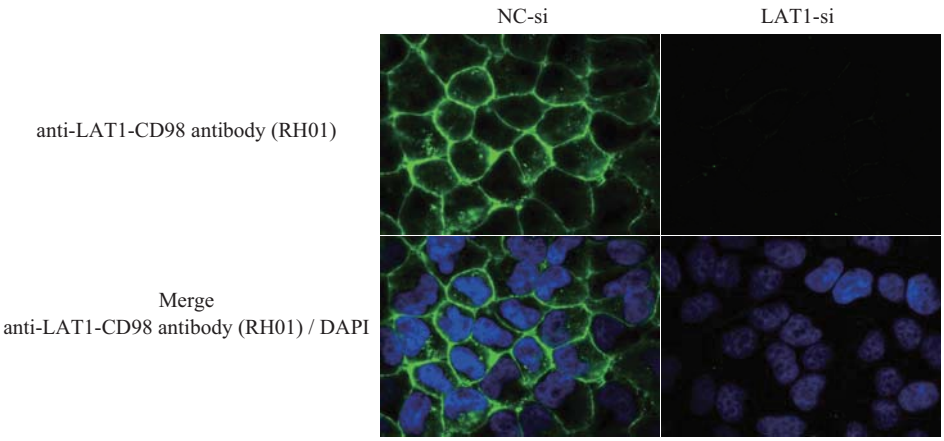

Fig 3. Immunofluorescence staining of LAT1-CD98 (green) in HeLa cells with anti-LAT1-CD98 antibody (5µg/mL).
- LAT1-si; LAT1 knockdown samples were reduced their signal.
- NC-si: non-targeting siRNA treated sample

Protocols;

Flow cytometry protocol (Cell Analyzing)

Cell Preparation

1. Remove cells from incubator.
2. Discard culture medium.
3. Briefly rinse the cell layer with PBS.
4. Add 0.25% trypsin-EDTA solution to dish. Return the dish to the incubator and incubate for 2-10 minutes or until cells are detached.
5. Resuspend cells in complete growth medium to inactivate the trypsin.

Staining

1. Aliquot $1x10^5$ cells into each assay tube.
2. Add 150 µL 0.2 % BSA in PBS to each tube and rinse by centrifugation.
3. Add 50 µL diluted primary antibody (5-20 µg/mL RH01 in 0.2 % BSA in PBS) to the assay tubes.
4. Incubate 45 minutes at 4 °C.
5. Add 100 µL 0.1 % BSA in PBS to each tube and wash by centrifugation.
6. Wash two times in 150 µL 0.1 % BSA in PBS by centrifugation.
7. Resuspend cells in 50 µL PE-labeled secondary antibody solution (Jackson Immuno Research 712-116-153), diluted 1:200 in 0.1 % BSA in PBS.
8. Incubate 30 minutes at 4 °C in the dark.
9. Add 100 µL0.1 % BSA in PBS to each tube and wash by centrifugation.
10. Wash two times in 150 µL 0.1 % BSA in PBS by centrifugation.
11. Resuspend cells in 100 µL PBS.
12. Add 100 µL Propidium Iodide (SIGMA, P4864), diluted 1:500 in PBS, to stain dead cells.
13. Analyze using flow cytometry.

Immunofluorescence Protocol

Cell Preparation

1. Grow cultured cells on Cell Chamber Slide.
2. Remove cells from incubator.
3. Discard medium and rinse briefly with PBS.
4. Fix in 4% paraformaldehyde in PBS for 15 minutes at room temperature.
5. Remove fixative and rinse three times in PBS for 3 minutes each.

Staining

1. Block sample in 3% BSA in PBS for 30 minutes at room temperature.
2. Remove excess blocking solution and apply diluted primary antibody (5 µg/µL RH01 in 0.2 % BSA in PBS).
3. Incubate 1 hour at room temperature.
4. Wash three times in PBS for 3 minutes each.
5. Apply Alexa Fluor 488 anti-rat IgG secondary antibody solution (Invitrogen, A11006), diluted 1:400 in 0.2 % BSA in PBS.
6. Incubate 1 hour at room temperature in the dark.
7. Wash three times in PBS for 3 minutes each.
8. Mount the coverslip using an anti-fade mounting reagent with DAPI (Invitrogen, S36939).
9. Seal slide by painting around edges of coverslip with nail polish.
10. Store slides flat at 4 °C protected from light until examined.

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