Anti-Pan-Lactyl-lysine Antibody [PSH03-73]

HA722037

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Species independent
Applications:	WB, IHC-P, IF-Cell, FC, Dot Blot
Clone number:	PSH03-73

Description: Post-translational modifications (PTMs) represent a crucial means of regulating diverse biological processes and cellular physiology by influencing protein structure and function. Histone lysine lactylation (Kla) is a newly discovered histone modification, which regulates gene expression in macrophages. In M1 macrophages, lactate is derived from incompletely oxidized glucose and then generate lactyl-CoA, which is transferred to lysine tails of histone proteins via the acetyl transferase p300. This modification was high in gene promoter regions that lack acetylation and associated with activation of genes expression. As a large number of studies have demonstrated that lysine acylation possessed a diverse range of substrate proteins, however, no systematic analysis has been reported for lysine lactylation.

Immunogen: Synthetic Lactyl lysine-containing peptide.

Positive control: HeLa treated with 100mM Lactate sodium for 24 hours cell lysate, human breast cancer tissue, human colon cancer tissue, mouse small intestine tissue, rat small intestine tissue, HeLa cells treated with 100mM Lactate sodium for 24 hours.

Subcellular location: Nucleus.

Recommended Dilutions:

WB	1:2,000
IHC-P	1:500-1:1,000
IF-Cell	1:1,000
FC	1:1,000
Dot Blot	1:2,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

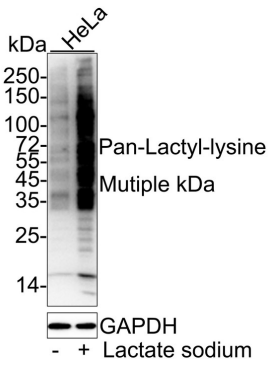

Fig1: Western blot analysis of Pan-Lactyl-lysine on different lysates with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100mM Lactate sodium for 24 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Observed band size: Mutiple kDa

Exposure time: 59 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722037) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

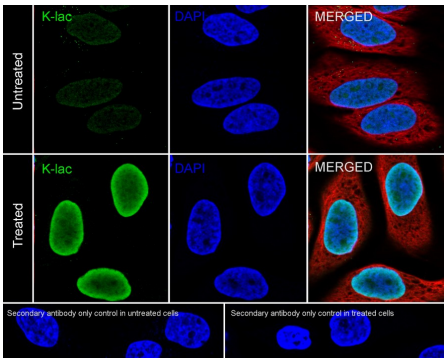

Fig2: Immunocytochemistry analysis of HeLa cells treated with or without 100mM Lactate sodium for 24 hours labeling Pan-Lactyl-lysine with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

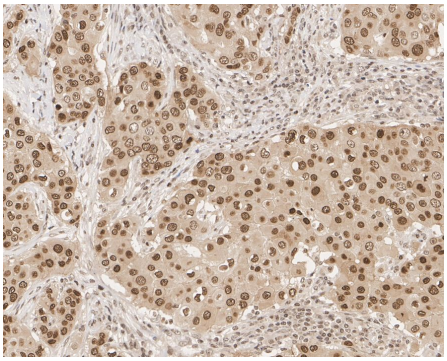

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722037) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

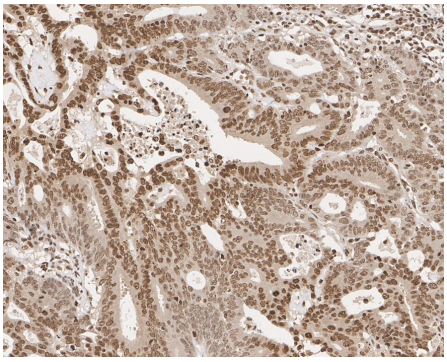

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722037) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

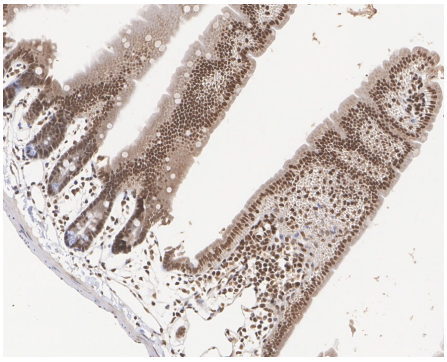

Fig5: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722037) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

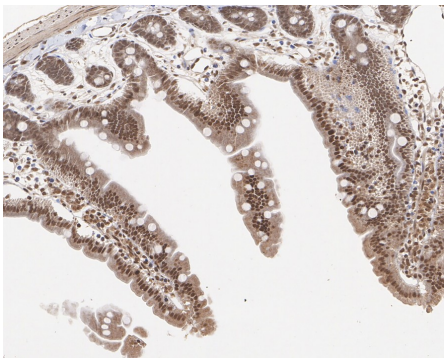

Fig6: Immunohistochemical analysis of paraffin-embedded rat small intestine tissue with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722037) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

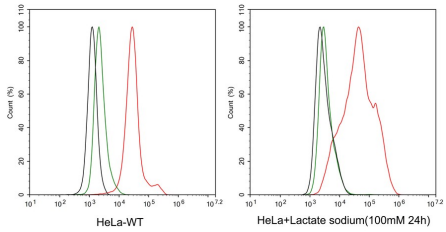

Fig7: Flow cytometric analysis of HeLa cells treated with or without 100mM Lactate sodium for 24 hours labeling Pan-Lactyl-lysine.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722037, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

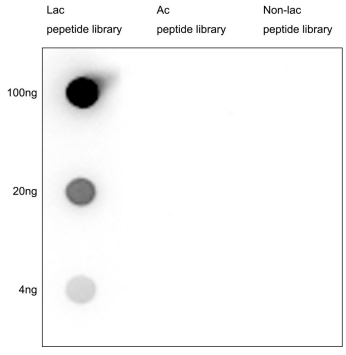

Fig8: Dot blot analysis of Pan-Lactyl-lysine on different proteins with Rabbit anti-Pan-Lactyl-lysine antibody (HA722037) at 1/2,000 dilution. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution for 1 hour at room temperature.

Lane 1: Lac pepetide library (positive)
Lane 2: Ac peptide library (negative)
Lane 3: Non-lac peptide library (negative)

Proteins loading: 100ng, 20ng, 4ng;

Blocking and dilution buffer: 5% NFDM/TBST;

Exposure time: 20 seconds.

Background References

1. Walsh, C. T., Garneau-Tsodikova, S., and Gatto, G. J. Jr. (2005). Protein posttranslational modifications: the chemistry of proteome diversifications. Angew. Chem. Int. Ed. Engl. 44, 7342–7372.
2. Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Hu, R., et al. (2019). Metabolic regulation of gene expression by histone lactylation. Nature 574, 575–580.

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation