PTM BIO
Pioneer in Proteomics
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Anti-acetyllysine antibody conjugated agarose beads

PTM BIO
Pioneer in Proteomics
Web: www.ptm-biolab.com.cn
Serveice: service@ptm-biolab.com
Support: 400-100-1145

Cat#: PTM-104 **Pack Size:** 0.5 mL
Species Reactivity: All species expected

Formulation:
0.25 mL settled agarose beads supplied as 50% slurry containing 66% glycerol.

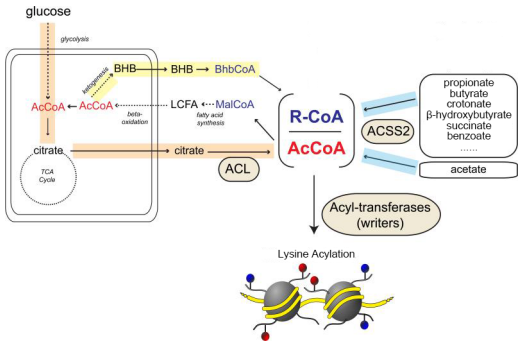
Application:
Peptide immunoaffinity enrichment followed by Mass Spectrometry-based proteomics; Protein immunoprecipitation.

Storage & Stability:
Store at -20°C and avoid freezing. Stable for 12 months from date of receipt.

Usage Recommendation:
20 µl settled beads per 2 mg peptides, totally 12 preps.

For research use only, not for therapeutic or diagnostic purposes in humans or animals

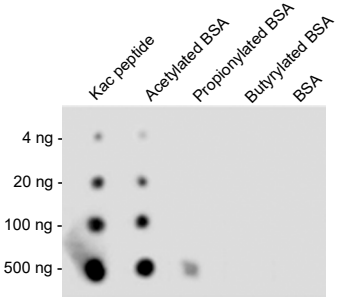
Scientific Background: Acylation is the addition of a short-chain acyl group to the amino group on the side chain of lysine residue. Beyond the widely studied **lysine acetylation**, nine types of lysine acylations have recently been identified on histones or non-histones: **propionylation, butyrylation, 2-hydroxyisobutyrylation, succinylation, malonylation, glutarylation, crotonylation, β-hydroxybutyrylation and benzoylation.** These post translational modifications (PTMs) are structurally different from each other, and exhibit distinct influence on protein functions. The acylations are regulated by the metabolism of different acyl-CoA forms, and catalyzed by lysine acyltransferase (KAT) to transfer acyl group to protein lysine residues.



Metabolic regulation of lysine acylations

Product Description: A mixture of high quality rabbit-derived polyclonal and mouse-derived monoclonal anti-acetyllysine antibodies are cross-linked to beaded agarose with stable covalent linkages. With the combinative advantages of polyclonal and monoclonal antibodies in global proteomic profiling of lysine acetylation, the product presents extraordinary capability to specifically capture the peptides/proteins bearing lysine acetylated residues.

Specificity: With the immobilization of highly specific anti-acetyllysine antibody, the acetyllysine antibody conjugated agarose beads selectively capture peptides/proteins bearing acetyllysine residues, but not cross-react with the peptides/proteins bearing structurally similar propionyllysine or butyryllysine residues.



Dot blot analysis of anti-acetyllysine antibody on different acylation proteins.

Lysine acetylation of histone or non-histone proteins plays a vital role in regulating biological functions including gene expression, DNA repair, DNA replication, apoptosis, energy metabolism etc. The affinity chromatography with anti-acetyllysine antibody conjugated agarose beads followed by MS/MS identification is viewed as the most preferable and efficient method for systematical screening of lysine acetylated proteins.

Performance of anti-acetyllysine antibody conjugated agarose beads in published studies

Samples	Proteomic summary	Reference
MEF cells	4623 Kac sites in 1800 Kac proteins	Yue Chen et al. ***Molecular & Cellular Proteomics***, 2012
Escherichia Coli	1070 Kac sites in 349 Kac proteins	Kai Zhang et al. ***Journal of Proteome Research***, 2013
Candida albicans	1073 Kac sites in 477 Kac proteins	Xiaowei Zhou et al. ***Journal of Proteome Research***, 2016
Anther of *Arabidopsis*	1354 Kac sites in 676 Kac proteins	Xiaojing Li et al. ***The Plant Journal***, 2017
Phaeodactylum tricornutum	2324 Kac sites in 1220 Kac proteins	Zhou Chen et al. ***Molecular & Cellular Proteomics***, 2017
Seedling of *Oryza sativa*	1353 Kac sites in 866 Kac proteins	Chao Xue et al. ***Proteomics***, 2018

Related Products:

PTM-104K-Kac peptide enrichment kit	Cat#: PTM-104K
Anti-acetyllysine mouse mAb (clone Kac-01)	Cat#: PTM-101
Anti-acetyllysine rabbit pAb	Cat#: PTM-105
Anti-succinyllysine antibody conjugated agarose beads	Cat#: PTM-402
Anti-crotonyllysine antibody conjugated agarose beads	Cat#: PTM-503
Anti-2-hydroxyisobutyryllysine antibody conjugated agarose beads	Cat#: PTM-804

Peptide immunoaffinity enrichment with Antibody Beaded Agarose

1. Mix bead suspension and aliquot 40 µl 50% bead slurry to 0.6 ml tube.

2. Wash beads with 0.5 ml pre-chilled PBS. Spin down beads at 1000 x g for 1 min at 4°C, and remove the supernatants. Repeat twice.

3. Dissolve 2 mg peptides in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, 0.5% Nonidet P-40, pH 8.0).

4. Remove any possible precipitates in peptide solution by centrifuging at 12,000 x g for 10 min at 4°C;

5. Mix peptide solution with pre-washed antibody conjugated beads. Incubate at 4°C for 4h with gentle shaking.

6. Harvest beads by centrifuging at 1000 x g for 1 min at 4°C.

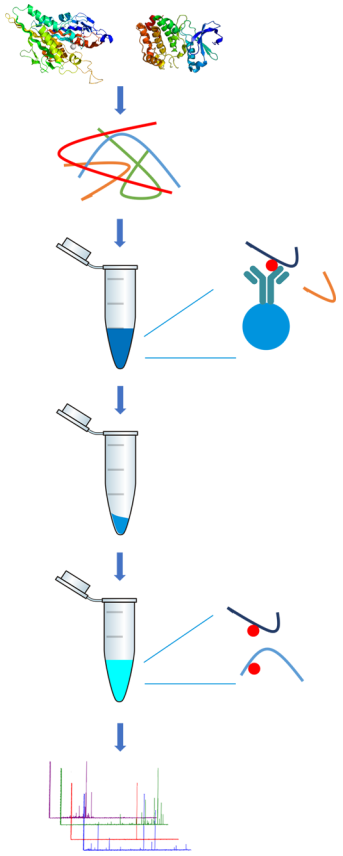
7. Wash the beads four times with 1 ml of NETN buffer and twice with deionized water.

8. Elute bound peptides with 1% trifluoroacetic acid. Repeat twice and combine all three elutes.

(Chen, Y., 2012, *Molecular & Cellular Proteomics*)

NOTE:
Optimal results could be obtained by using PTM-104K Kac peptide enrichment kit (PTM Bio, Inc.).

Outline of peptide immunoaffinity enrichment



(1) Extract proteins from samples.

(2) Digest proteins to peptide.

(3) Dissolve the peptide with NETN buffer, add proper amount of settled antibody beads, incubate at 4°C.

(4) Pellet the beads, Wash with NETN Buffer and deionized water step by step.

(5) Elute the bound peptides with 1% trifluoroacetic acid.

(6) Identify & Quantify peptide by LC-MS/MS

Reference:

1. Chen, Y., et al. (2012). Quantitative Acetylome Analysis Reveals the Roles of SIRT1 in Regulating Diverse Substrates and Cellular Pathways. ***Molecular & Cellular Proteomics*** 11, 1048–1062.

2. Hebert, A.S., et al. (2013). Calorie Restriction and SIRT3 Trigger Global Reprogramming of the Mitochondrial Protein Acetylome. ***Molecular Cell*** 49, 186–199.

3. Du, Y., et al. (2015). Lysine Malonylation Is Elevated in Type 2 Diabetic Mouse Models and Enriched in Metabolic Associated Proteins. ***Molecular & Cellular Proteomics*** 14, 227–236.

4. Huang, H., et al. (2018). Landscape of the regulatory elements for lysine 2-hydroxyisobutyrylation pathway. ***Cell Research***. 28, 111–125.

5. Huang, H., et al. (2018). Lysine benzoylation is a histone mark regulated by SIRT2. ***Nature Communications*** 9, 3374.

Academic Platform

Technical Support