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Datasheet for 600-401-939

Lysine Acetylated Antibody

Overview

Description:	Anti-Lysine Acetylated (AcK) (RABBIT) Antibody - 600-401-939
Item No.:	600-401-939
Size:	100 µg
Applications:	WB, IP
Reactivity:	Broad
Host Species:	Rabbit

Product Details

Background:	Acetylation refers to the process of introducing an acetyl group into a compound. Proteins are typically acetylated on lysine residues and this reaction relies on acetyl-coenzyme A as the acetyl group donor. Acetylated Lysine antibody is useful in Cancer, Cell Biology and Chromatin & Nuclear Signaling research.
Synonyms:	acetyl Lysine antibody, Acetylated lysine antibody, Lysine antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Reactivity:	Broad
Immunogen Type:	Other
Immunogen:	Anti-Lysine Acetylated Antibody was prepared from whole rabbit serum produced by repeated immunizations with acetylated lysine conjugated KLH.
Purity/Specificity:	Anti-Lysine Acetylated Antibody was prepared from monospecific antiserum by immunoaffinity chromatography using acetylated lysine peptide coupled to agarose. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit IgG. The antibody reacts specifically with acetylated lysine residues.
Relevant Links:	• 600-401-939 SDS

Application Details

Tested Applications:	WB
Suggested Applications:	IP (Based on references)
Application Note:	Anti-Lysine Acetylated Antibody is suitable for use in ELISA, western blotting, immunofluorescence microscopy, and immunoprecipitation assays. Although not tested, this antibody is likely functional in RIA, flow cytometry, and immunohistochemistry.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:1,000-1:2,500
FC:	User Optimized
IF:	User Optimized
IHC:	User Optimized
IP:	10 µg/mg protein sample
WB:	1:500-1:1,000

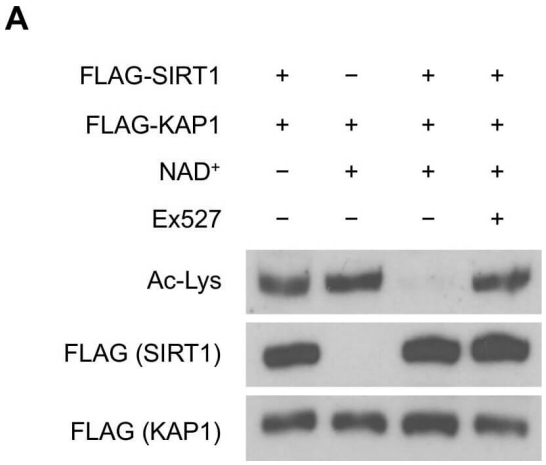
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	None
Stabilizer:	50% (v/v) Glycerol

Shipping & Handling

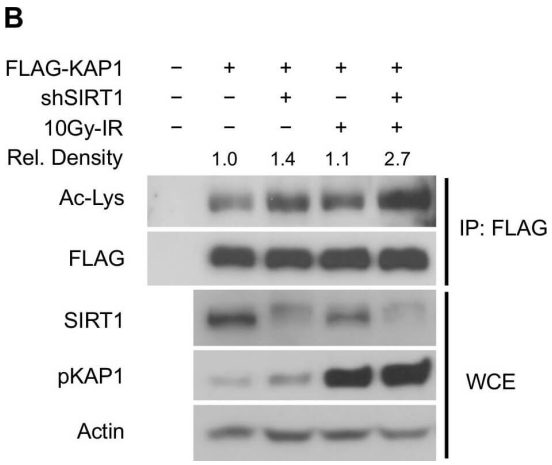
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot

SIRT1 deacetylates KAP1 in vitro and in vivo.(A) SIRT1 deacetylates KAP1 in vitro. Exogenous FLAG-tagged KAP1 and SIRT1 were purified by anti-FLAG immunoprecipitation. Combination of purified proteins was incubated in deacetylation buffer supplemented with or without NAD+ cofactor. Ex527 (40µM) was added to block the deacetylase activity of SIRT1. (B) SIRT1 deacetylates KAP1 in vivo. FLAG-tagged KAP1 was transfected into control or SIRT1 depleted HEK293T cells. Cells were treated with or without IR 1 hour before harvest. Cell lysates were subjected to immunoprecipitation using anti-FLAG antibody, followed by western blot analysis to assess the total KAP1 acetylation level. Relative density of the overall acetyl lysine was quantified using ImageJ software. Acetylation level was normalized to corresponding FLAG band. (C) HEK293T cells transfected with FLAG-tagged KAP1 were treated with DMSO or Ex527 (20µM) for 6 hours before harvest. Cell lysates were then immunoprecipitated with FLAG-conjugated agarose beads, and the immunoprecipitates were blotted with anti-acetyl lysine antibody to determine the total acetylation level. (D and E) HEK293T cells were transfected with FLAG-tagged KAP1 and treated with DMSO or Ex527 (20µM) for 6 hours before harvest. Recombinant KAP1 was purified and sent for mass spectrometric analysis. Acetyl residues with >2-fold enhancement after inhibitor treatment were considered to be SIRT1 targeted sites. (F) Site-directed mutagenesis was applied to generate 4KR mutant. FLAG-tagged WT-KAP1 or 4KR mutant were transfected into control or SIRT1 depleted HEK293T cells. Total acetylation levels of recombinant WT-KAP1 and 4KR mutant were assessed by immunoblotting. Figure provided by CiteAb. Source: PLoS One, PMID: 25905708.



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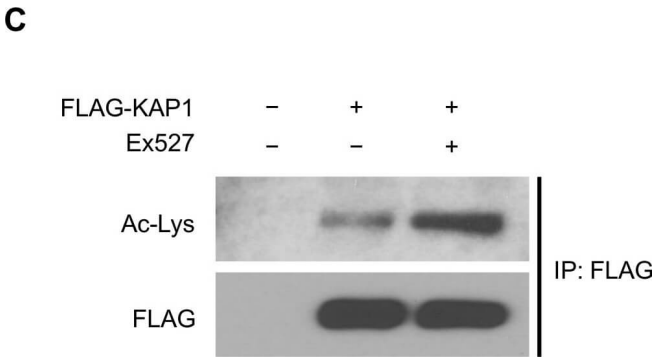

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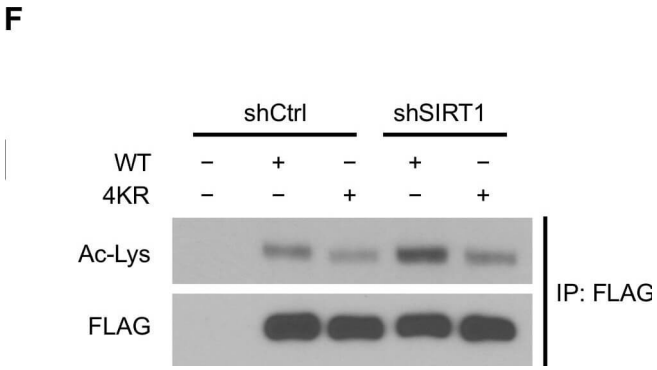

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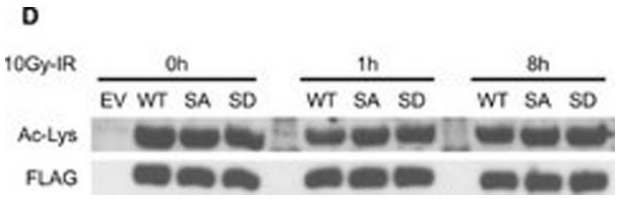
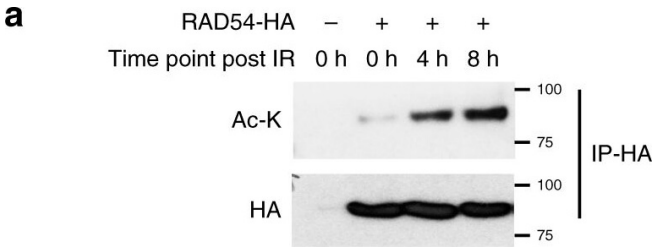


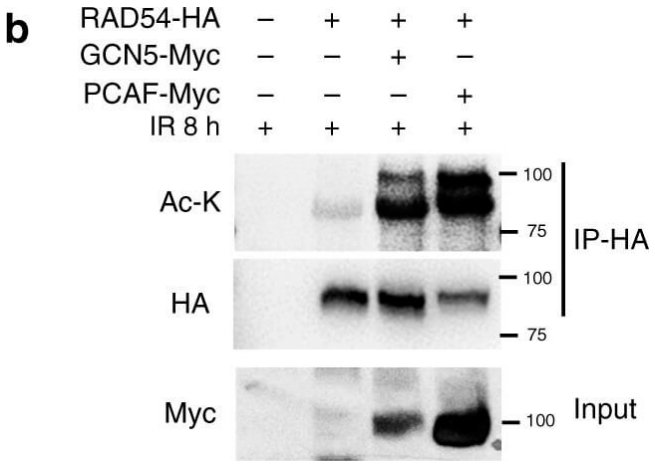
Western Blot

Acetylation does not crosstalk with IR-induced pSer824 and does not affect the co-repressor activity of KAP1.(A) Control and SIRT1 depleted HEK293T cells were harvested at indicated time points following 10Gy-IR. IR-induced KAP1 pSer824 was examined by phosphorylation specific antibody. (B) Cells transfected with FLAG-tagged WT-KAP1 or 4KR mutant were harvested at indicated time points following 4Gy-IR. Exogenous KAP1 was immunoprecipitated and pSer824 was examined by phosphorylation specific antibody. (C) Cyclin D3 luciferase construct was co-transfected with combinations of HA-tagged E2F1, FLAG-tagged KAP1, and 4KR mutant. Luciferase activity was measured 48 hours post transfection. (D) Cells transfected with FLAG-tagged WT-KAP1 or KAP1 phospho mutants (S824A and S824D) were harvested at indicated time points following 10Gy-IR. Recombinant KAP1 was purified by immunoprecipitation and the total acetylation level was assessed by immunoblotting. Figure provided by CiteAb. Source: PLoS One, PMID: 25905708.



Western Blot

RAD54 acetylation is important for BRD9 recognition and HR activity.a–c RAD54 is acetylated by GCN5/PCAF and deacetylated by HDAC 6/HDAC11 following induction of DNA damage. a 293T cells were transfected with control or RAD54-HA plasmid. Twenty-four hours after transfection, cells were exposed to the 10-Gy IR and harvested at the indicated time points. Immunoprecipitation with anti-HA beads was performed. Blots were probed with the indicated antibodies. b 293T cells were transfected with the indicated plasmids. Twenty-four hours after transfection, cells were exposed to 10-Gy IR, and lysates were collected after 8 h. Immunoprecipitation with anti-HA beads was performed. Blots were probed with the indicated antibodies. c 293T cells were transfected with the indicated plasmids, treated as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. d, e RAD54 K515 acetylation is important for RAD51–RAD54 complex formation. d 293T cells were transfected with the indicated plasmids, treated as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. e 293T cells were transfected with the indicated plasmids, exposed to either no IR or 10-Gy IR as indicated, and subjected to immunoprecipitation as outlined in b. Blots were probed with the indicated antibodies. f–h RAD54 K515 acetylation is essential for HR activity. f, g U2OS cells were transfected with the indicated plasmids and exposed to 2-Gy IR. Cells were fixed after 8 h and stained for the indicated proteins. Representative immunofluorescence images of RAD51 (green) and RAD54 (red) are shown in f. Quantification of the indicated foci is shown in g. Representative data (mean ± SEM) are shown from n = 50 cells examined over three independent experiments. **p < 0.01,***p < 0.001 by two-sided unpaired t test. NS not significant. Scale bar, 10 µm. h Survival curves of U2OS cells expressing the indicated constructs and exposed to the indicated doses of PARPi or cisplatin. Representative data (mean ± SEM) are shown from n = 3 biologically independent samples. *p < 0.05, ***p < 0.001 by two-sided unpaired t test. Figure provided by CiteAb. Source: Nat Commun, PMID: 32457312.



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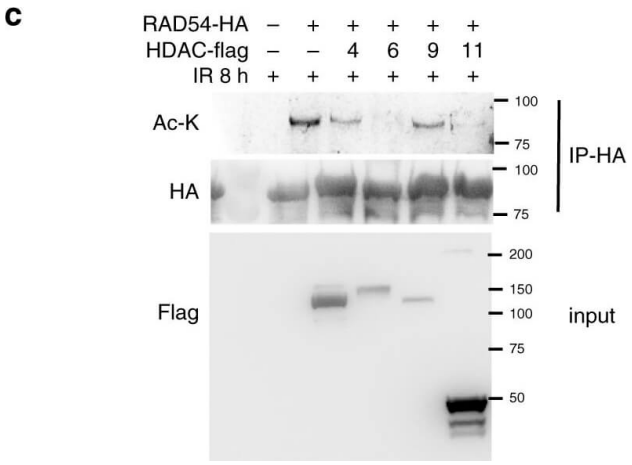

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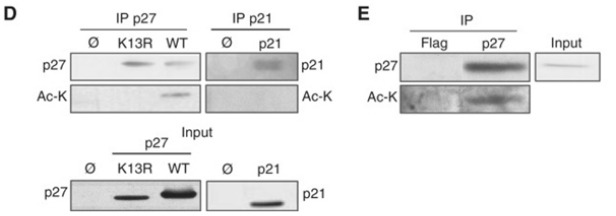
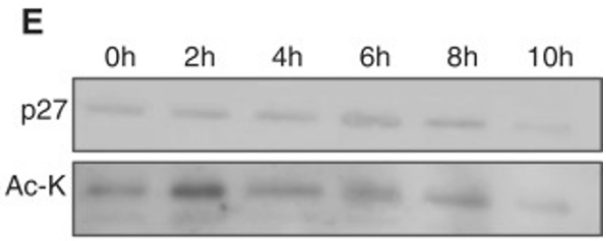
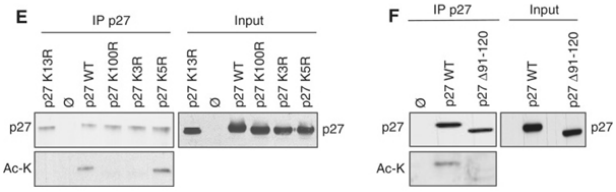


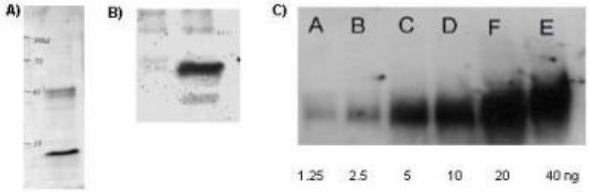
Immunoprecipitation
(D) HEK293T cells were cotransfected with PCAF plus p27WT or p27K13R, or transfected with an empty vector (ø). Then, cell extracts were subjected to IP with anti-p27. The levels of total or acetylated p27 were examined by WB with anti-p27 or anti-acetyl-lysine antibodies (Ac-K), respectively (upper left panel). A similar experiment was performed using p21 instead of p27 (upper right panel). Expression levels of p27 and p21 were examined by WB (bottom panels). (E) Cell extracts were subjected to IP with anti-p27 or anti-Flag (used as a control) and then the immunoprecipitates were analyzed by anti-p27 and anti-acetyl-Lysine antibodies (Ac-K). Fig 3. PMID: 22547391



Immunoprecipitation
(E) Extracts from HeLa cells at different times after cell-cycle resumption were subjected to IP with anti-p27. Then, the levels of p27 and those of acetylated p27 in the immunoprecipitates were assessed by WB with anti- p27 or with anti-acetyl-lysine (Ac-K), respectively. Fig 5.
PMID: 22547391



Western Incubation Box
(E) HEK293T cells were transfected with an empty vector (ø) or cotransfected with p27WT, p27-K13R, p27-K100R, p27-K3R or p27-K5R plus PCAF. Cell extracts were then subjected to IP with anti-p27 and the total levels of p27 or acetylated p27 were analyzed by WB with anti-p27 or anti acetyl-Lysine (Ac-K) antibodies (left panel). Expression levels of the different forms of p27 were determined by WB with anti-p27 (right panel). (F) Cells were transfected with an empty vector or cotransfected with p27WT or p27(Δ91–120) plus PCAF. Cell extracts were then subjected to IP with anti-p27 and the levels of p27 or acetylated p27 were analyzed by WB with anti-p27 or anti-acetyl-Lysine (Ac-K) antibodies (left panel). Expression levels of the different forms of p27 were determined by WB with anti-p27 (right panel). Fig 4.
PMID: 22547391



Western Blot
Rockland's Affinity Purified anti-Acetylated Lysine (AcK) antibody is shown to detect acetylated histone in TSA-treated mouse spleen cell lysate (Panel A); control (left lane) and TSA-treated mouse spleen cell lysate (right lane) in panel B; and in acetylated BSA loaded as indicated (panel C).

References

- Zhou Q et al. The bromodomain containing protein BRD-9 orchestrates RAD51–RAD54 complex formation and regulates homologous recombination-mediated repair. *Nat Commun.* (2020)
- Noritsugu K et al. Identification of zinc finger transcription factor EGR2 as a novel acetylated protein. *Biochem Biophys Res Commun.* (2017)
- Lin et al. KAP1 Deacetylation by SIRT1 Promotes Non-Homologous End-Joining Repair. *PLOS One* (2015)
- Perez-Luna M et al. PCAF regulates the stability of the transcriptional regulator and cyclin-dependent kinase inhibitor p27 Kip1. *Nucleic Acids Res.* (2012)

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