abcam

Product datasheet

Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade ab217876



RabMAb

6 References 画像数8

製品の概要

製品名 Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade

製品の詳細 Rabbit monoclonal [EPR21146] to KAT2A / GCN5 - ChIP Grade

由来種 Rabbit

アプリケーション 適用あり: ChIP, WB, IP 種交差性 交差種: Mouse. Human

交差が予測される動物種: Rat 🔷

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HAP1, HEK-293T, MCF7 and HeLa whole cell lysate; human fetal brain tissue lysate; His-

tagged human KAT2A / GCN5 recombinant protein (aa86-336); Wild-type U-2 OS and NIH/3T3

cell lysates. IP: HeLa whole cell lysate.

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル **ウローン名** EPR21146

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab217876の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ChIP		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 94 kDa (predicted molecular weight: 94 kDa).
IP		1/30.

ターゲット情報

機能 Functions as a histone acetyltransferase (HAT) to promote transcriptional activation. Acetylation

of histones gives a specific tag for epigenetic transcription activation. Has significant histone acetyltransferase activity with core histones, but not with nucleosome core particles. In case of HIV-1 infection, it is recruited by the viral protein Tat. Regulates Tat's transactivating activity and may help inducing chromatin remodeling of proviral genes. Component of the ATAC complex, a

complex with histone acetyltransferase activity on histones H3 and H4.

組織特異性 Expressed in all tissues tested, with most abundant expression in ovary.

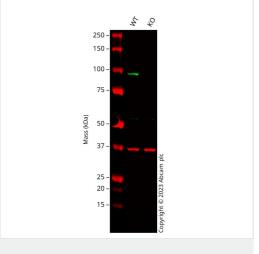
配列類似性 Belongs to the GCN5 family.

Contains 1 bromo domain.

Contains 1 N-acetyltransferase domain.

細胞内局在 Nucleus.

画像



Western blot - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876)

All lanes : Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) at 1/1000 dilution

Lane 1: Wild-type U-2 OS cell lysate

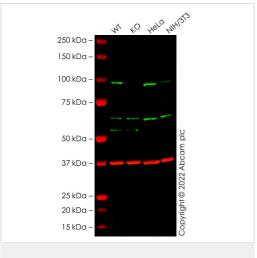
Lane 2: GCN5 knockout U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 94 kDa **Observed band size:** 94 kDa

Western blot: Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab217876 was shown to bind specifically to KAT2A / GCN5. A band was observed at 94 kDa in wild-type U-2 OS cell lysates with no signal observed at this size in kat2a knockout cell line. To generate this image, wildtype and kat2a knockout U-2 OS cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) **All lanes :** Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) at 1/1000 dilution

Lane 1: Wild-type U-2 OS cell lysate

Lane 2: GCN5 knockout U-2 OS cell lysate

Lane 3 : HeLa cell lysate
Lane 4 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

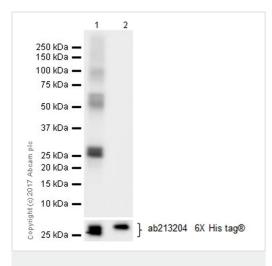
Secondary

All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 94 kDa **Observed band size:** 95 kDa

False colour image of Western blot: Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab217876 was shown to bind specifically to KAT2A / GCN5. A band was observed at 95 kDa in wild-type U-2 OS cell lysates with no signal observed at this size in kat2a knockout cell line. To generate this image, wild-type and kat2a knockout U-2 OS cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) **All lanes :** Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) at 1/1000 dilution

Lane 1 : His-tagged human KAT2A / GCN5 recombinant protein (aa86-336), 10 ng

Lane 2 : His-tagged human KAT2B recombinant protein (aa74-326), 10 ng

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Developed using the ECL technique.

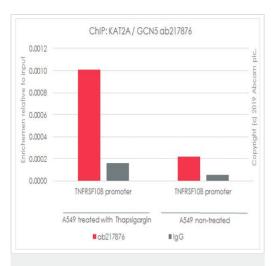
Predicted band size: 94 kDa **Observed band size:** 27 kDa

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 1 second

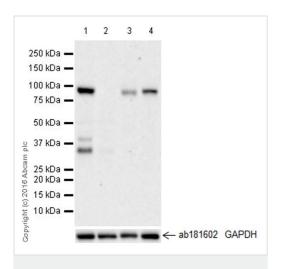
The blot was developed on a BIO-RAD® ChemiDoc™ MP

instrument.



ChIP - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876)

Immunoprecipitation - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876)



Western blot - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876)

Chromatin was prepared from A549 treated with thapsigargin (1µM 12 hours) cells according to the Abcam Dual X-ChIP protocol*.

Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab217876 (red), and 20 μ l of Protein A/G sepharose beads. 5 μ g of rabbit normal lgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources? keywords=X%20ChIP%20protocol

KAT2A / GCN5 was immunoprecipitated from 0.35mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab217876 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab217876 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10ug (Input).

Lane 2: ab217876 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab217876 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.

All lanes : Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) at 1/1000 dilution

Lane 1: Wild type HAP1 whole cell lysate

Lane 2: KAT2A / GCN5-knockout HAP1 whole cell lysate whole cell lysate

Lane 3: HEK-293T (uman epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 4: MCF7 (human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 94 kDa **Observed band size:** 94 kDa

Exposure time: 3 minutes

ab217876 was shown to specifically react with KAT2A / GCN5 in wild-type HAP1 cells as signal was lost in KAT2A / GCN5 knockout cells. Wild-type and KAT2A / GCN5 knockout samples were subjected to SDS-PAGE. Ab217876 and <u>ab181602</u> (Human anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/200000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) secondary antibody at 1/100000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrumentusing the ECL technique.

250 kDa -250 kDa -150 kDa -100 kDa -150 kDa -75 kDa -100 kDa -75 kDa -50 kDa -50 kDa 🕳 37 kDa -37 kDa -25 kDa -25 kDa -20 kDa -20 kDa -15 kDa -15 kDa -10 kDa -10 kDa -

Western blot - Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) **All lanes :** Anti-KAT2A / GCN5 antibody [EPR21146] - ChIP Grade (ab217876) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: Human fetal brain tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Lane 2: VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

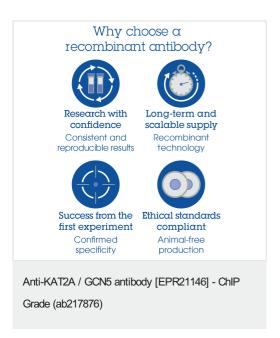
Predicted band size: 94 kDa Observed band size: 94 kDa

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: Lane 1: 15 seconds; Lane 2: 3 minutes

The blot was developed on a BIO-RAD® ChemiDoc $^{\mathsf{TM}}$ MP

instrument.



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