abcam

Product datasheet

Anti-KAT1 / HAT1 antibody [EPR18775] ab194296





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Overview

Product name Anti-KAT1 / HAT1 antibody [EPR18775]

Description Rabbit monoclonal [EPR18775] to KAT1 / HAT1

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa, MCF7, F9, LLC1, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Human

> fetal liver and fetal kidney lysates; Mouse thymus lysate; Mouse and rat colon, brain, kidney and spleen lysates. IHC-P: Human tonsil, Human gastric adenocarcinoma, mouse colon and rat colon tissues. ICC/IF: HeLa and F9 cells. Flow Cyt (intra): HeLa cells. IP: HeLa and F9 whole cell

lysates.

General notes 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**.

Properties

Form Liquid

Storage instructions 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.

Storage buffer

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number

EPR18775

Isotype

lgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab194296 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/150.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 49 kDa).
ICC/IF		1/500.
IP		1/50.

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Function

Acetylates soluble but not nucleosomal histone H4 at 'Lys-5' (H4K5ac) and 'Lys-12' (H4K12ac) and, to a lesser extent, acetylates histone H2A at 'Lys-5' (H2AK5ac). Has intrinsic substrate specificity that modifies lysine in recognition sequence GXGKXG. May be involved in nucleosome assembly during DNA replication and repair as part of the histone H3.1 and H3.3 complexes. May play a role in DNA repair in response to free radical damage.

Sequence similarities

Belongs to the HAT1 family.

Developmental stage

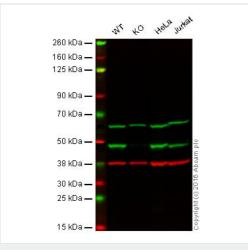
Highly expressed in mitotic cells (at protein level).

Cellular localization

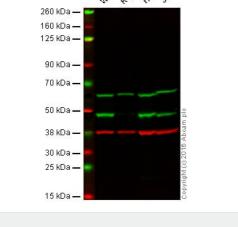
Nucleus matrix and Cytoplasm. Nucleus. Nucleus matrix. Nucleus > nucleoplasm. Localization is predominantly nuclear in normal cells. Treatment with hydrogen peroxide or ionizing radiation

enhances nuclear localization through redistribution of existing protein.

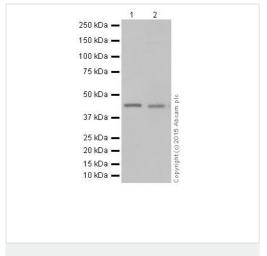
Images



[EPR18775] (ab194296)



Western blot - Anti-KAT1 / HAT1 antibody



Western blot - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

Lane 1: Wild-type HAP1 cell lysate (40 µg)

Lane 2: KAT1/HAT1 knockout HAP1 cell lysate (40 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: Jurkat cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab194296 observed at 48 kDa. Red - loading control, ab18058, observed at 37 kDa.

ab194296 was shown to recognize KAT1/HAT1 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when KAT1/HAT1 knockout samples were examined. Wild-type and KAT1 / HAT1 knockout samples were subjected to SDS-PAGE. Ab194296 and ab18058 (loading control to GAPDH) were diluted at 1/1000 and 1/10,000 dilution respectively and incubated overnight at 4°C. Blots were developed with IRDye® 800CW Goat anti-Rabbit lgG (H + L) and IRDye® 680 Goat anti-Mouse IgG (H + L) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

All lanes: Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296) at 1/1000 dilution

Lane 1: Human fetal liver lysate

Lane 2: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

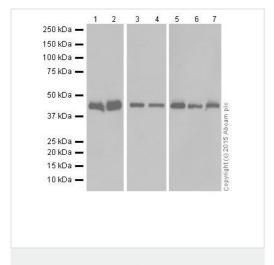
Secondary

All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 49 kDa Observed band size: 45 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296) **All lanes :** Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296) at 1/1000 dilution

Lane 1: Mouse colon lysate

Lane 2: Rat colon lysate

Lane 3: HeLa (Human epithelial cells from cervix

adenocarcinoma) whole cell lysate

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole

cell lysate

Lane 5: F9 (Mouse embyro testicular cancer cell line) whole cell

lysate

Lane 6: LLC1 (Mouse lung carcinoma cell line) whole cell lysate

Lane 7: Mouse thymus lysate

Lysates/proteins at 20 µg per lane.

Secondary

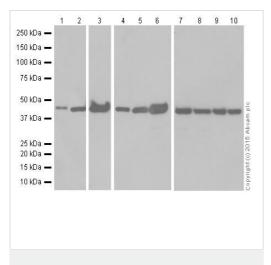
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 49 kDa **Observed band size:** 45 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 3 minutes; Lane 3 and 4: 2 seconds;

Lane 5, 6 and 7: 5 seconds



Western blot - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296) **All lanes :** Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296) at 1/1000 dilution

Lane 1: Mouse brain lysate

Lane 2: Mouse kidney lysate

Lane 3: Mouse spleen lysate

Lane 4: Rat brain lysate

Lane 5: Rat kidney lysate

Lane 6: Rat spleen lysate

Lane 7: C6 (Rat glial tumor cells) whole cell lysate

Lane 8: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysate

Lane 9: PC-12 (Rat adrenal gland pheochromocytoma) whole cell

lysate

Lane 10: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

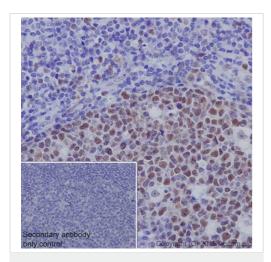
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 49 kDa **Observed band size:** 45 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1, 2, 4, 5 and 6: 3 minutes; Lane 3: 1 minute;

Lane 7, 8, 9 and 10: 5 seconds.

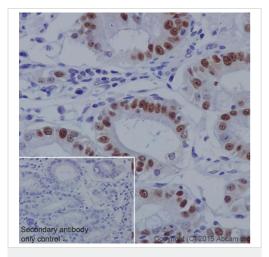


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAT1 / HAT1 antibody
[EPR18775] (ab194296)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on the germinal center of Human tonsil is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

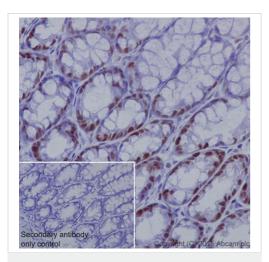


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAT1 / HAT1 antibody
[EPR18775] (ab194296)

Immunohistochemical analysis of paraffin-embedded Human gastric adenocarcinoma tissue labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on cancer cells of Human gastric adenocarcinoma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

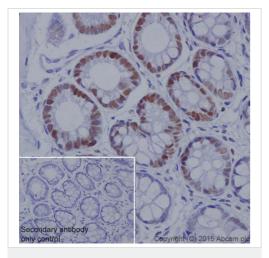


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAT1 / HAT1 antibody
[EPR18775] (ab194296)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on epithelial cells of mouse colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

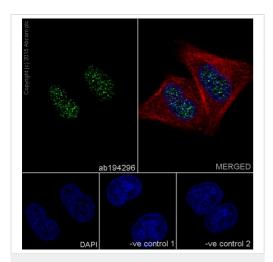


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAT1 / HAT1 antibody
[EPR18775] (ab194296)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on epithelial cells of rat colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

ab194296 MERGED

DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

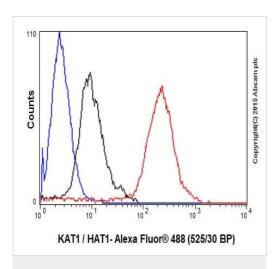
The negative controls are as follows:

-ve control 1: ab194296 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized F9 (Mouse embyro testicular cancer cell line) cells labeling KAT1 / HAT1 with ab194296 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on F9 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

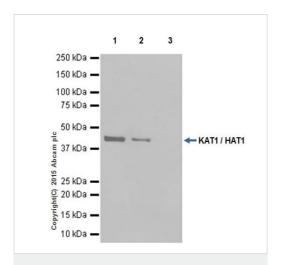
The negative controls are as follows:

-ve control 1: ab194296 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling KAT1 / HAT1 with ab194296 at 1/150 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

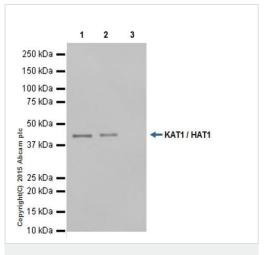
KAT1 / HAT1 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab194296 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab194296 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

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

Lane 2: ab194296 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab194296 in HeLa whole cell lysate.

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



Immunoprecipitation - Anti-KAT1 / HAT1 antibody [EPR18775] (ab194296)

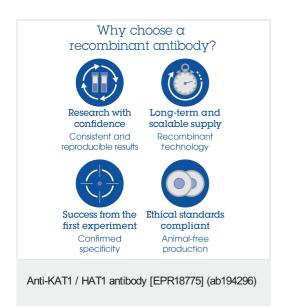
KAT1 / HAT1 was immunoprecipitated from 1mg of F9 (Mouse embyro testicular cancer cell line) whole cell lysate with ab194296 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab194296 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: F9 whole cell lysate 10ug (Input).

Lane 2: ab194296 IP in F9 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab194296 in F9 whole cell lysate.

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



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