

Anti-HDAC2 Antibody [SY29-02] - BSA and Azide free

HA750134



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 55 kDa
Clone number:	SY29-02

Description: Histone deacetylase 2 (HDAC2) is an enzyme that in humans is encoded by the HDAC2 gene. It belongs to the histone deacetylase class of enzymes responsible for the removal of acetyl groups from lysine residues at the N-terminal region of the core histones (H2A, H2B, H3, and H4). As such, it plays an important role in gene expression by facilitating the formation of transcription repressor complexes and for this reason is often considered an important target for cancer therapy. Though the functional role of the class to which HDAC2 belongs has been carefully studied, the mechanism by which HDAC2 interacts with histone deacetylases of other classes has yet to be elucidated. HDAC2 is broadly regulated by protein kinase 2 (CK2) and protein phosphatase 1 (PP1), but biochemical analysis suggests its regulation is more complex (evinced by the coexistence of HDAC1 and HDAC2 in three distinct protein complexes). HDAC2 has been shown to play a role in cardiac hypertrophy, Alzheimer's disease, Parkinson's disease, acute myeloid leukemia (AML), osteosarcoma, and stomach cancer.

Immunogen: Synthetic peptide within Human HDAC2 aa 439-488 / 488.

Positive control: HeLa cell lysate, HEK-293 cell lysate, MCF7 cell lysate, Jurkat cell lysate, L-929 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, HeLa, HEK-293, NIH/3T3, human tonsil tissue, mouse spinal cord tissue, rat spinal cord tissue, C6.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q92769 Human | P70288 Mouse | F7ENH8 Rat

Recommended Dilutions:

WB	1:20,000-1:50,000
IF-Cell	1:100-1:1,000
IF-Tissue	1:200-1:1,000
IHC-P	1:6,000
FC	1:1,000
IP	1-2µg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

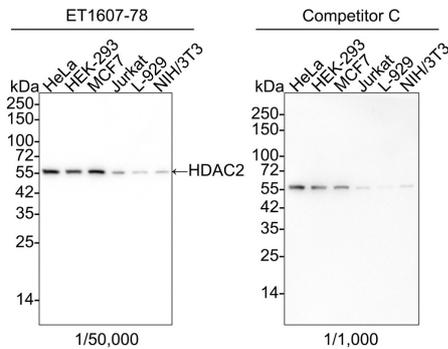
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Images

Fig1: Western blot analysis of HDAC2 on different lysates with Rabbit anti-HDAC2 antibody (HA750134) at 1/50,000 dilution and competitor's antibody at 1/1,000 dilution.



Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: L-929 cell lysate
Lane 6: NIH/3T3 cell lysate

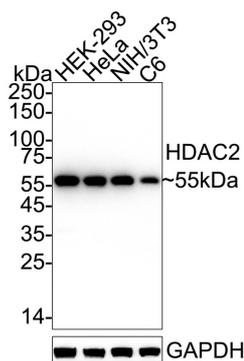
Lysates/proteins at 15 µg/Lane.

Predicted band size: 55 kDa
Observed band size: 55 kDa

Exposure time: Lane 1-6 (left):30 seconds; Lane 1-6 (right): 1 minute 20 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA750134) at 1/50,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of HDAC2 on different lysates with Rabbit anti-HDAC2 antibody (HA750134) at 1/20,000 dilution.



Lane 1: HEK-293 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)

Predicted band size: 55 kDa
Observed band size: 55 kDa

Exposure time: 10 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA750134) at 1/20,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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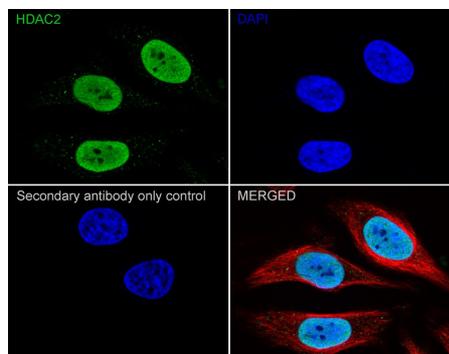
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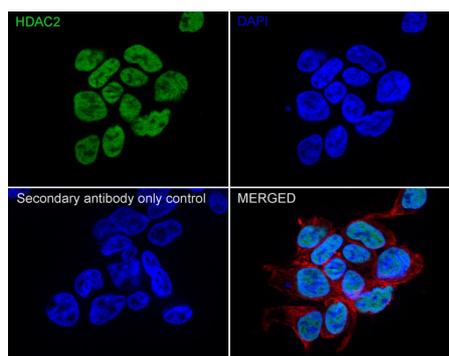
Fig3: Immunocytochemistry analysis of HeLa cells labeling HDAC2 with Rabbit anti-HDAC2 antibody (HA750134) at 1/1,000 dilution.



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-HDAC2 antibody (HA750134) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

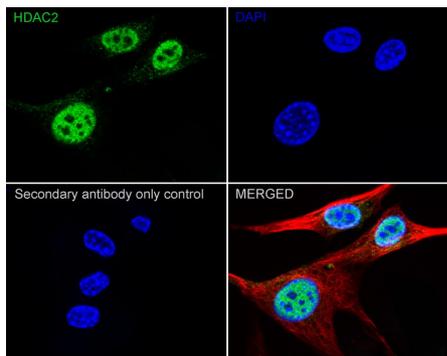
Fig4: Immunocytochemistry analysis of HEK-293 cells labeling HDAC2 with Rabbit anti-HDAC2 antibody (HA750134) at 1/100 dilution.



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-HDAC2 antibody (HA750134) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

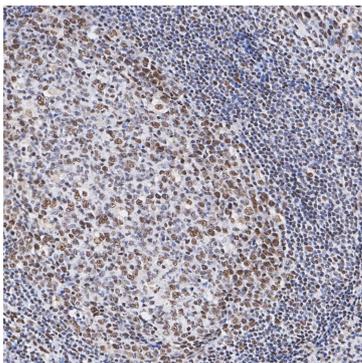
Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling HDAC2 with Rabbit anti-HDAC2 antibody (HA750134) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-HDAC2 antibody (HA750134) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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.

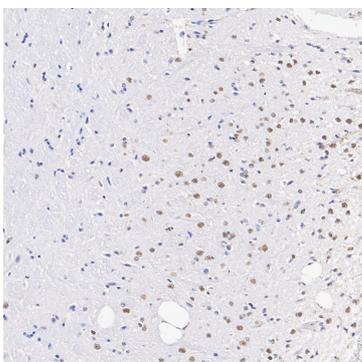
Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-HDAC2 antibody (HA750134) at 1/6,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750134) at 1/6,000 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.

Fig7: Immunohistochemical analysis of paraffin-embedded mouse spinal cord tissue with Rabbit anti-HDAC2 antibody (HA750134) at 1/6,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750134) at 1/6,000 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.

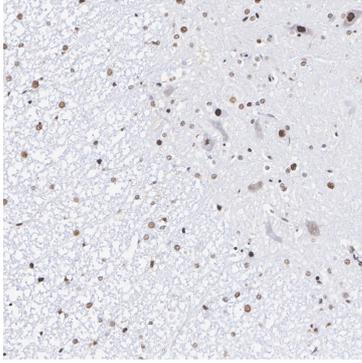


Fig8: Immunohistochemical analysis of paraffin-embedded rat spinal cord tissue with Rabbit anti-HDAC2 antibody (HA750134) at 1/6,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750134) at 1/6,000 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.

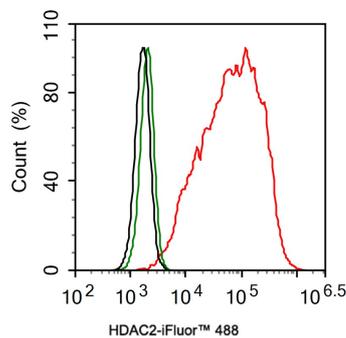


Fig9: Flow cytometric analysis of HeLa cells labeling HDAC2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750134, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

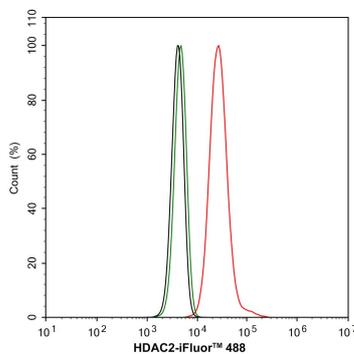


Fig10: Flow cytometric analysis of NIH/3T3 cells labeling HDAC2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750134, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

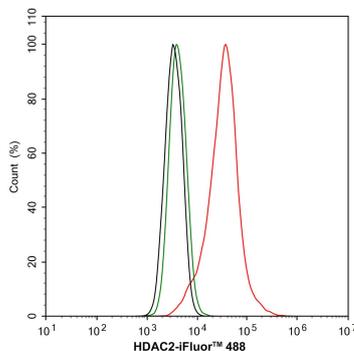


Fig11: Flow cytometric analysis of C6 cells labeling HDAC2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750134, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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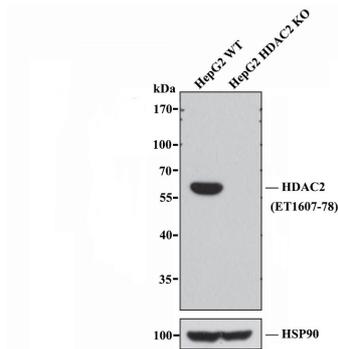


Fig12: All lanes: Western blot analysis of HDAC2 with anti-HDAC2 antibody [SY29-02] (HA750134) at 1:1,000 dilution.

Lane 1: Wild-type HepG2 whole cell lysate (20 µg).

Lane 2: HDAC2 knockout HepG2 whole cell lysate (20 µg).

HA750134 was shown to specifically react with HDAC2 in wild-type HepG2 cells. No band was observed when HDAC2 knockout samples were tested. Wild-type and HDAC2 knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-HDAC1 antibody (HA750134, 1/1,000) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

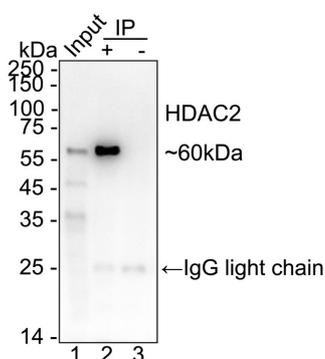


Fig13: HDAC2 was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA750134 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750134 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)

Lane 2: HA750134 IP in NIH/3T3 cell lysate

Lane 3: Rabbit IgG instead of HA750134 in NIH/3T3 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)

Exposure time: 7 seconds; ECL: K1801

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Mould AW et al. Blimp1/Prdm1 Functions in Opposition to Irf1 to Maintain Neonatal Tolerance during Postnatal Intestinal Maturation. *PLoS Genet* 11:e1005375 (2015).
2. Yahiro, K. et al. 2012. Low-density lipoprotein receptor-related protein-1 (LRP1) mediates autophagy and apoptosis caused by *Helicobacter pylori* VacA. *J. Biol. Chem.* 287: 31104-31115.

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