

Basic Information

Product Name	Anti-HDAC3 Antibody	
Gene Name	HDAC3	
Source	Rabbit	
Isotype	IgG	
Species Reactivity	human, mouse, rat	
Tested Application	WB, IHC, IHC-F, ICC/IF, FCM	
Contents	500 ug/ml antibody with PBS , 0.02% NaN ₃ , 1 mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HDAC3(411-428aa NEFYDGDHDNDKESDVEI), identical to the related rat and mouse sequences.	
concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	49KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry in paraffin section (IHC): 1:50-400 Immunohistochemistry in frozen section (IHC-F): 1:50-400 Immunocytochemistry/Immunofluorescence(ICC/IF): 1:50-400 (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

HDAC3(HISTONE DEACETYLASE 3) is a member of the histone deacetylase/acuc/apha family of proteins that is an enzyme that in humans is encoded by the HDAC3 gene. The HDAC3 gene is mapped to 5q31.3. HDAC3 has histone deacetylase activity and represses transcription when tethered to a promoter. It may participate in the regulation of transcription through its binding with the zinc-finger transcription factor YY1. The protein can also down-regulate p53 function and thus modulate cell growth and apoptosis. And this gene is regarded as a potential tumor suppressor gene. HDAC3 has an open reading frame of 428 amino acids and shares 53% amino acid identity with HDAC1 and 52% with HDAC2. The catalytic domain of HDAC4 interacts with HDAC3 via the transcriptional corepressor NCOR2. All experimental conditions leading to the suppression of HDAC4 binding to NCOR2 and to HDAC3 resulted in loss of enzymatic activity associated with HDAC4. HDAC3 recruitment to the genome displays a circadian rhythm in mouse liver.

Selected Validation Data

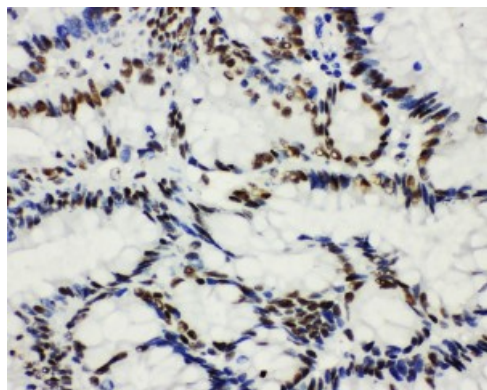


Figure 1. IHC analysis of HDAC3 using anti-HDAC3 antibody (BA0916).

HDAC3 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-HDAC3 Antibody (BA0916) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

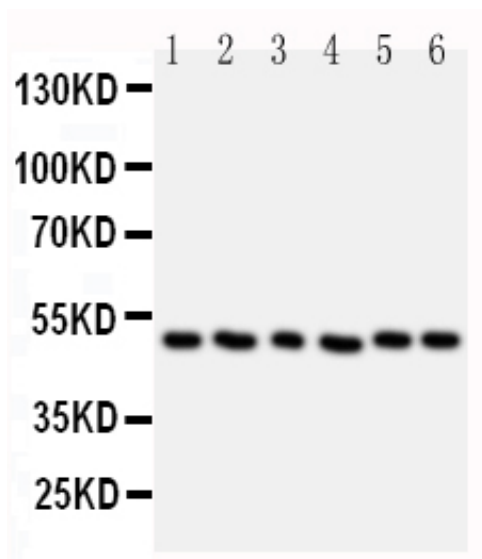


Figure 6. Western blot analysis of HDAC3 using anti-HDAC3 antibody (BA0916).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 μ g of sample under reducing conditions.

Lane 1: Rat Stomach Tissue Lysate

Lane 2: Rat Testis Tissue Lysate

Lane 3: MCF-7 Cell Lysate

Lane 4: HELA Cell Lysate

Lane 5: JURKAT Cell Lysate

Lane 6: SKOV Cell Lysate

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-HDAC3 antigen affinity purified polyclonal antibody (Catalog # BA0916) at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system.

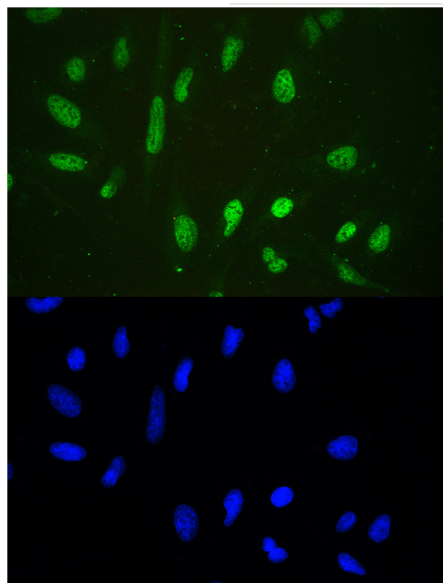


Figure 7. IF analysis of HDAC3 using anti- HDAC3 antibody (BA0916).

HDAC3 was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 μ g/mL rabbit anti-HDAC3 Antibody (BA0916) overnight at 4°C. DyLight488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

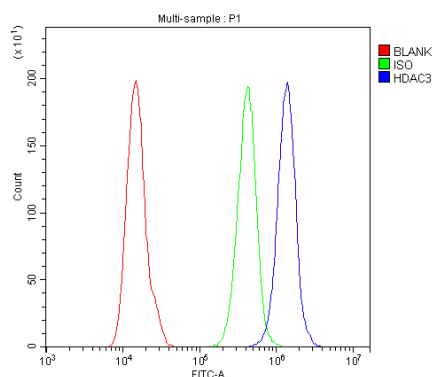


Figure 9. Flow Cytometry analysis of A431 cells using anti-HDAC3 antibody (BA0916).

Overlay histogram showing A431 cells stained with BA0916 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti- HDAC3 Antibody (BA0916, 1 μ g/1x10⁶ cells) for 30 min at 20°C. DyLight488 conjugated goat anti-rabbit IgG (BA1127, 5-10 μ g/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 μ g/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.