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antibody and ELISA experts

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Anti-Brd4 Rabbit Monoclonal Antibody

Catalog Number: M00123

Overview

Product Name	Anti-Brd4 Rabbit Monoclonal Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Brd4 Rabbit Monoclonal Antibody catalog # M00123. Tested in WB, IHC, ICC/IF, IP applications. This antibody reacts with Human, Mouse, Rat.
Application	IP, IF, IHC, ICC, WB
Clonality	Monoclonal DBH-2
Formulation	Rabbit IgG in stabilizing components, phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	O60885

Technical Details

Immunogen	A synthesized peptide derived from human Brd4
Isotype	Rabbit IgG
Form	Liquid
Concentration	0.5mg/ml
Purification	Affinity-chromatography
Suggested Dilutions	WB 1:500-2000 IHC 1:50-200 ICC/IF 1:50-200 IP 1:20

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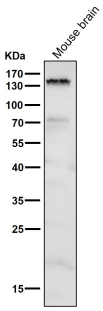
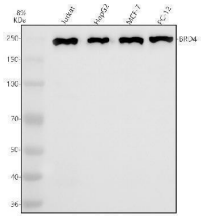
Anti-Brd4 Rabbit Monoclonal Antibody (M00123) Images



Western blot analysis of Brd4 using anti-Brd4 antibody (M00123). Electrophoresis was performed on a 8% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: mouse brain tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-Brd4 antigen affinity purified monoclonal antibody (M05837-1) at 1:500 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 1:500 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for Brd4 at approximately 240 kDa. The expected band size for Brd4 is at 152 kDa.



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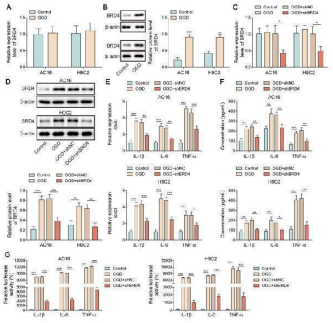

O-GlcNAcylation of BRD4 inhibited NF-kappaB p65-mediated transcription of pro-inflammatory cytokines. (A)&(B) The expression of BRD4 in OGD-exposed cardiomyocytes was detected by RT-qPCR and Western blotting. H9C2 and AC-16 cells were transfected with shBRD4, and then subjected to OGD. (C)&(D) RT-qPCR and Western blotting analysis of BRD4 mRNA and protein levels. (E)&(F) The mRNA levels and concentrations of TNF-alpha, IL-1beta, and IL-6 were determined by RT-qPCR and ELISA. (G) The binding of NF-kappaB p65 to TNF-alpha, IL-1beta, and IL-6 promoters was confirmed by dual-luciferase reporter assay. (H)&(I) Co-IP assay verified the exogenous and endogenous interplay between OGT and BRD4 proteins. (J) O-GlcNAcylation of

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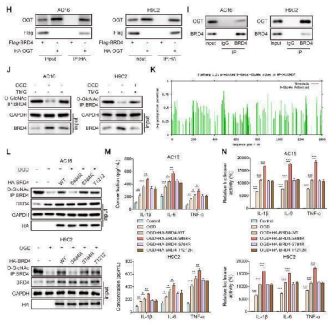
BRD4 protein in OGD-stimulated cardiomyocytes was evaluated. (K) YinOYang database predicated the potential O-GlcNAc sites on BRD4. OGD-challenged H9C2 and AC-16 cells were transfected with BRD4 WT plasmid or BRD4 plasmids with mutant O-GlcNAc sites (BRD4-S484R, BRD4-S784R, and BRD4-T1212R). (L) O-GlcNAcylation of BRD4 protein in H9C2 and AC-16 cells was detected. (M) Concentrations of TNF-alpha, IL-1beta, and IL-6 were detected by ELISA. (N) The interaction between NF-kappaB p65 and TNF-alpha, IL-1beta, and IL-6 promoters was validated by dual-luciferase reporter assay. n=3 for A-N. Student's t test (for A, B) and one-way ANOVA (for C-G, M, N) were performed to analyze data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.Index in PubMed under a CC BY license. PMID: 40585977



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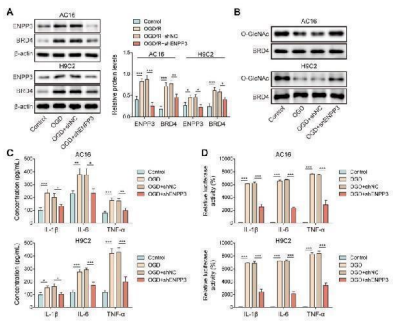

ENPP3 contributed to inflammation by inhibiting O-GlcNAcylation of BRD4. H9C2 and AC-16 cells were transfected with shENPP3, followed by exposure to OGD. (A) ENPP3 and BRD4 protein levels were measured by Western blotting. (B) The O-GlcNAc level of BRD4 protein was assessed. (C) The production of TNF-alpha, IL-1beta, and IL-6 was determined by ELISA. (D) Dual-luciferase reporter assay evaluated the binding of NF-kappaB p65 to TNF-alpha, IL-1beta, and IL-6 promoters. n=3 for A-D. One-way ANOVA was performed to analyze data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.Index in PubMed under a CC BY license. PMID: 40585977

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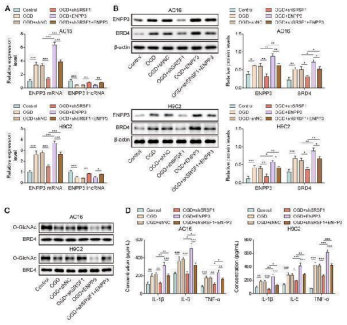
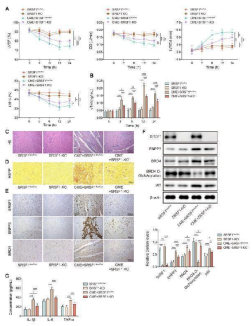
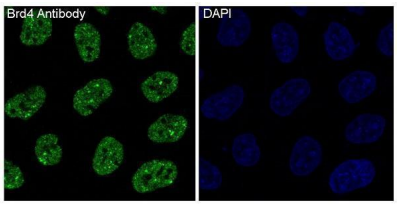


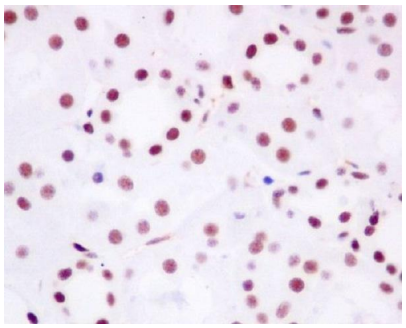
SRSF1/ENPP3 axis suppressed BRD4 O-GlcNAcylation to promote inflammation in CME. The OGD-stimulated cardiomyocytes were transfected with shSRSF1, ENPP3 overexpression plasmid, or a combination of them. (A) ENPP3 mRNA and lncRNA ENPP3 expression levels were detected by RT-qPCR. (B) The protein abundance of ENPP3 and BRD4 was assessed by Western blotting. (C) The O-GlcNAc level of BRD4 was determined. (D) ELISA was carried out to measure TNF-alpha, IL-1beta, and IL-6 concentrations. n=3 for A-D. One-way ANOVA was performed to analyze data. * p < 0.05, ** p < 0.01, *** p < 0.001.Index in PubMed under a CC BY license. PMID: 40585977



Myocardium-specific SRSF1 knockout alleviated CME-induced inflammation via inactivation of the ENPP3/BRD4/NF-kappaB pathway. SRSF1 flox/flox and SRSF1-KO rats were injected with microspheres into the left ventricle to induce CME. (A) LVEF, LVFS, LVEDd, and CO were detected to evaluate cardiac function. (B) The serum cTnI level in different groups was measured by ELISA. (C) Pathological alterations in myocardial tissues were observed by HE staining (scale bar = 100 um). (D) Myocardial infarct size was measured by HBFP staining (scale bar = 100 um). (E) SRSF1, ENPP3, and BRD4 expression in myocardial tissues was evaluated by immunohistochemical staining (scale bar = 100 um). (F) The protein abundance of SRSF1, ENPP3, BRD4, p65, and O-GlcNAcylation of BRD4 was detected by Western blotting or Co-IP, respectively. (G) ELISA was carried out to measure TNF-alpha, IL-1beta, and IL-6 concentrations. n=6 for A-G. ANOVA for repeated measurement (for A, B), and one-way ANOVA (for F, G) was performed to analyze data. * p < 0.05, ** p < 0.01, *** p < 0.001.Index in PubMed under a CC BY license. PMID: 40585977



Immunofluorescent analysis of Hela cells, using Brd4 Antibody .



Immunohistochemical analysis of paraffin-embedded human kidney, using Brd4 Antibody.

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