

POLYCLONAL ANTIBODY

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Catalog No. 70-230EX

Anti Brg1 antibody, rabbit polyclonal, affinity-purified

Key words: BAF complex, Chromatin remodeling complex, CREBBP, CREST-BRG1 complex, CYP27B1 gene, c-Fos promoter, Nuclear hormone receptor, Neural stem cell, Neuron, NR2B promoter, phospho-RB1-HDAC repressor complex, Transcriptional coactivator, Vitamin D receptor, WINAC complex, Zeb1,

Function: Brg1 (1647 aa, 185 kDa) is transcriptional coactivator cooperating with nuclear hormone receptors to potentiate transcriptional activation. Component of the CREST-BRG1 complex, a multiprotein complex that regulates promoter activation by orchestrating a calcium-dependent release of a repressor complex and a recruitment of an activator complex. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC repressor complex. Upon calcium influx, RB1 is dephosphorylated by calcineurin, which leads to release of the repressor complex. At the same time, there is increased recruitment of CREBBP to the promoter by a CREST-dependent mechanism, which leads to transcriptional activation. The CREST-BRG1 complex also binds to the NR2B promoter, and activity-dependent induction of NR2B expression involves a release of HDAC1 and recruitment of CREBBP. Belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and the neuron-specific chromatin remodeling complex (nBAF complex). During neural development a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. The transition from proliferating neural stem/progenitor cells to post-mitotic neurons requires a switch in subunit composition of the npBAF and nBAF complexes. As neural progenitors exit mitosis and differentiate into neurons, npBAF complexes which contain ACTL6A/BAF53A and PHF10/BAF45A, are exchanged for homologous alternative ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C subunits in neuron-specific complexes (nBAF). The npBAF complex is essential for the self-renewal/proliferative capacity of the multipotent neural stem cells. The nBAF complex along with CREST plays a role regulating the activity of genes essential for dendrite growth. SMARCA4/BAF190A may promote neural stem cell self-renewal/proliferation by enhancing Notch-dependent proliferative signals, while concurrently making the neural stem cell insensitive to SHH-dependent differentiating cues By similarity. Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene. Acts as a corepressor of ZEB1 to regulate E-cadherin transcription and is required for induction of epithelial-mesenchymal transition (EMT) by ZEB1



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Applications

- 1) Western blotting (1/1,000 dilution) (Fig. 1)
- 2) Immunoprecipitation (Assay dependent) (Fig. 2)
- 3) Immunofluorescence staining (1/1,000 dilution) (Fig. 3)

Not tested for other applications.

Immunogen: C-terminal 50 amino acids of human BRG1. C-KLGRKEKAQDRLK GGRRRPSR GSRAKPVVSDDDSEEEQEEDRSGSGSEED, conjugated with KLH

Reactivity: Human and mouse. Not tested in other species

Product: Affinity-purified from rabbit antiserum with immunogen peptide conjugated with agarose beads.

Form: 1 mg/ml in PBS (x1), 50% glycerol. Filter-sterilized. Azide and carrier free.

Size: 100ug

Storage: Sent at 4°C. Upon arrival, spin-down and store at -20°C.

Data link: uniprot/P51532 human Brg1

Publication: This antibody has been described and used in the following publication.

Nishimoto N. et al (2012) Heterocomplex formation by Arp4 and β-actin is involved in the integrity of the Brg1 chromatin remodeling complex. J Cell Sci.125: 3870-82. pubmed/Brg1 Free article. WB, IP

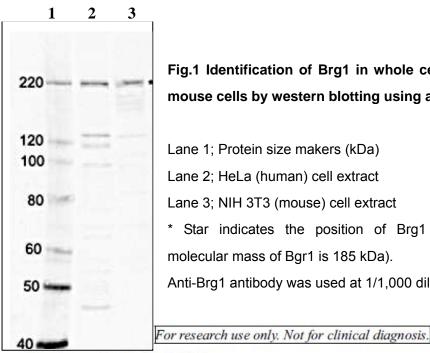


Fig.1 Identification of Brg1 in whole cell extracts of human and mouse cells by western blotting using anti-Brg1 antibody.

Lane 1; Protein size makers (kDa)

Lane 2; HeLa (human) cell extract

Lane 3; NIH 3T3 (mouse) cell extract

* Star indicates the position of Brg1 protein bands (Predicted molecular mass of Bgr1 is 185 kDa).

Anti-Brg1 antibody was used at 1/1,000 dilution

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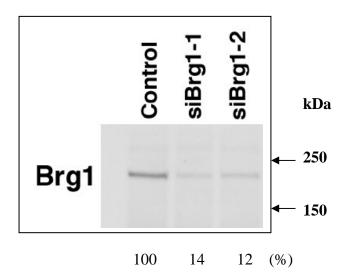
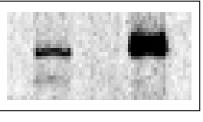


Fig.2 Silencing of Brg1 expression by specific siRNA for Brg1.

HeLa cells were transfected with the indicated siRNAs. Forty-eight hours after transfection, whole cell lysates were analyzed by western blotting. The signal intensities of the bands were quantified and shown below the blot, with control siRNA-treated cells as 100 %.



1 2 3

Fig 3. Immunoprecipitation of Brg1 protein from nuclear extracts of HeLa cells by using anti-Brg1 antibody.

Brg1 was precipitated with anti-Brg1 antibody and proteinG-conjugated agarose beads and probed with anti-Brg1 antibody by western blotting..

Lane1; Input nuclear extracts

Lane 2; Control IP with non-immune IgG

Lane 3; Immunoprecipitation with anti-Brg1 antibody

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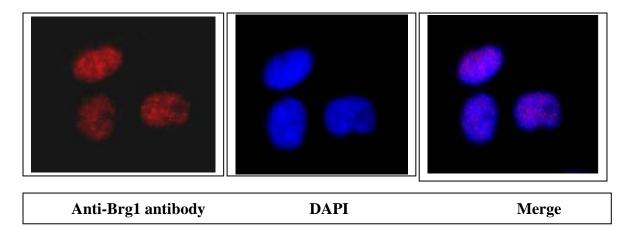


Fig.4. Immunofluorescence staining of Brg1 protein in HeLa cells.

Hela cells were fixed in 4% paraformaldehyde overnight and permeabilized in 0.25% TritonX 100 in PBS for 10 min. Anti-Brg1 antibody was used at 1/1,000 dilution.

As second antibody, goat anti-rabbit IgG conjugated with Alex488 was used at 1/1,000 dilution. Nuclei were stained with DAPI.

Brg1 protein is localized in nucleus.

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