# **Product Datasheet**

# Alkaline Phosphatase/ALPP Antibody (8B6) - BSA Free NB110-3638SS

Unit Size: 0.025 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.





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# NB110-3638SS

Alkaline Phosphatase/ALPP Antibody (8B6) - BSA Free

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Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	8B6
Preservative	0.05% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein A purified
Buffer	PBS
Target Molecular Weight	70 kDa
Product Description	
Host	Mouse
Gene ID	250
Gene Symbol	ALPP
Species	Human, Mouse, Rat
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 28197547). Rat reactivity reported in scientific literature (PMID: 30599898).
Specificity/Sensitivity	Alkaline Phosphatase, Placental - both Regan and Nagao isoenzymes. No cross reactivity with other isoenzymes of Alkaline Phosphatase.
Immunogen	Hep-2 cells with boosted surface expression of Alkaline Phosphatase, Placental. [UniProt# P05187].
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Radioimmunodiffusion
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry reported in multiple pieces of scientific literature, ELISA 1:100-1:2000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry- Paraffin 1:100-1:200, Immunohistochemistry-Frozen 1:10-1:500, Radioimmunodiffusion
Application Notes	In WB a band can be seen at ~70 kDa. For IHC, Proteolytic Induced Epitope Retrieval (PIER) is required.



#### Images

Immunocytochemistry/Immunofluorescence: Alkaline Phosphatase/ALPP В Antibody (8B6) [NB110-3638] - Representative montage images are shown for control dissociated DRG culture. (B) Dissociated DRGs cultures exposed to conditioned medium from alkaline phosphataseexpressing rat GRPs. Image collected and cropped by CiteAb from the following publication (eneuro.org/content/4/1/ENEURO.0171-16.2017), licensed under a CC-BY license. Immunocytochemistry/Immunofluorescence: Alkaline Phosphatase/ALPP Antibody (8B6) [NB110-3638] - analysis of ALPP in MDA-MB-231 cells using an anti-ALPP antibody (blue - cell membrane, green - ALPP). Image from verified customer review. Western Blot: Alkaline Phosphatase/ALPP Antibody (8B6) [NB110-3638] 250> - Analysis of Alkaline Phosphatase (Placental) expression in JAR whole 150> cell lysate. 100> 75> 50> 37> 25> 15> 10> Immunohistochemistry-Paraffin: Alkaline Phosphatase/ALPP Antibody (8B6) [NB110-3638] - Alkaline Phosphatase, Placental Antibody (8B6) [NB110-3638] - IHC staining of Alkaline Phosphatase (Placental) in human placenta using DAB with hematoxylin counterstain. Proteolytic Induced Epitope Retrieval (PIER) was used.



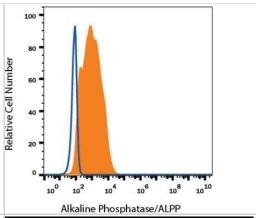
Flow Cytometry: Alkaline Phosphatase/ALPP Antibody (8B6) [NB110-3638] - Detection of Alkaline Phosphatase/ALPP in Human HeLa Cell Line by Flow Cytometry. Human HeLa cell line was stained with Mouse Anti- Alkaline Phosphatase/ALPP Monoclonal Antibody (Catalog # NB110-3638, filled histogram), or Mouse IgG2A isotype control (Catalog # MAB003, open histogram) followed by APC-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).

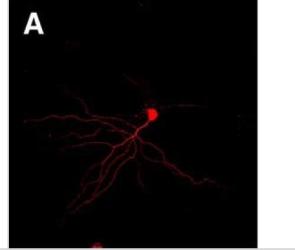
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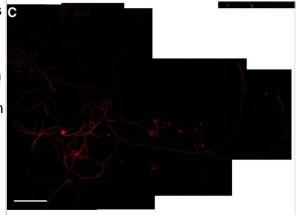
TIP30 was regulated by HIF-2 $\alpha$  at protein level in HCC cell lines. Lentivirus infection was used to establish a stable knocking-down or overexpression of HIF-2 $\alpha$  in MHCC97H cell lines. The relationship between HIF-2 $\alpha$  and TIP30 was detected by Western blot. a Knockingdown of HIF-2 $\alpha$  upregulated TIP30 expression. b Knocking-down of TIP30 did not influence the expression of HIF-2 $\alpha$ . c, d Metformin in combination with sorafenib synergistically inhibited HIF-2 $\alpha$  protein expression and subsequently upregulated TIP30 protein expression. e MHCC97H cells were exposed to CoCl2 (400  $\mu$ M) for 6 h, and anti-HIF-2 $\alpha$  or anti-IgG was used for immunoprecipitation. Immunoprecipitated and purified DNA together with 1 % of input DNA were used for PCR amplification of a 214-bp product encompassing HRE region of TIP30 promoter Image collected and cropped by CiteAb from the following open publication (https://www.jhoonline.org/content/9/1/20), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

KDM1A is strongly overexpressed in human medulloblastomas, cell lines 🖸 derived from them and murine medulloblastic tumors. a Data from a representative cohort of 62 medulloblastomas (MB) and normal cerebellar tissue (CB) used in a published microarray analysis [22,23] were re-analyzed for KDM1A expression. \*\*\*p < 0.0001 b KDM1A protein expresion was evaluated immunohistochemically in a tissue microarray of 70 medulloblastomas (MB) and 9 tissue samples of normal cerebellum (CB). Micrograph showing KDM1A-positive staining in a representative MB sample, and KDM1A-negative staining in CB, scale bar = 100  $\mu$ m. c Bars reflect the proportion of cells with strong (black), moderate (dark grey), weak (light grey) or no (white) nuclear KDM1A staining. A twotailed student's t-test revealed a significant upregulation of KDM1A protein in the medulloblastomas represented in the tissue microarrays. \*\*\*p < 0.0001 d Bars represent KDM1A expression measured using realtime RT-PCR and normalized to the geometric mean of GAPDH, UBC and HPRT expression in a panel of human medulloblastoma cell lines derived from diverse histological tumor subtypes and the SK-N-BE human neuroblastoma cell line, known to express high levels of KDM1A as a reference. e Bars represent KDM1A expression measured using real-time RT-PCR in medulloblastic tumors (black) spontaneously arising in genetically engineered mice with activating mutations in the sonic hedgehog pathway, SmoA1 MB (p = 0.014) and Ptch+/- MB (p = 0.037), compared to normal murine cerebellum (CB, white). f Strong KDM1A protein expression was confirmed in the medulloblastic tumors from SmoA1- and Ptch+/--mice relative to KDM1A expression in cerebellar tissue (CB) using western blotting of tissue lysates. β-actin expression was used as a loading control. Image collected and cropped by CiteAb from the following open publication

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#### **Publications**

Chen Y, Hong M, Xu H et al. EGFR inhibition in lung adenocarcinoma upregulates cell surface expression of the placental antigen ALPP and enhances efficacy of ALPP-ADC therapy bioRxiv 2023-03-29 (ICC/IF, WB)

Qiu M, Li C, Cai Z et al. 3D Biomimetic Calcified Cartilaginous Callus that Induces Type H Vessels Formation and Osteoclastogenesis Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2023-03-31 [PMID: 36999832] (IHC-P, Rat)

Ontawong A, Duangjai A, Srimaroeng C Coffea arabica bean extract inhibits glucose transport and disaccharidase activity in Caco 2 cells Biomed Rep 2021-08-18 [PMID: 34405045]

Chan YH, Ho KN, Lee YC et al. Melatonin enhances osteogenic differentiation of dental pulp mesenchymal stem cells by regulating MAPK pathways and promotes the efficiency of bone regeneration in calvarial bone defects Stem cell research & therapy 2022-02-19 [PMID: 35183254] (WB)

Kim J, Singh A, DelPoeta M et al. The effect of sterol structure upon clathrin-mediated and clathrin-independent endocytosis. J Cell Sci. [PMID: 28655854] (Human)

Ontawong A, Duangjai A, Muanprasat C et al. Lipid-lowering effects of Coffea arabica pulp aqueous extract in Caco-2 cells and hypercholesterolemic rats. Phytomedicine 2018-06-01 [PMID: 30599898] (WB, Human, Rat)

Goulao M, Ghosh B, Urban MW et al. Astrocyte progenitor transplantation promotes regeneration of bulbospinal respiratory axons, recovery of diaphragm function, and a reduced macrophage response following cervical spinal cord injury Glia 2018-12-11 [PMID: 30548313] (IF/IHC, Human)

Merianda TT, Jin Y, Kalinski AL et al. Neural Progenitor Cells Promote Axonal Growth and Alter Axonal mRNA Localization in Adult Neurons. eNeuro. 2017-02-15 [PMID: 28197547] (ICC/IF, Mouse)

Kiem HP, Andrews RG, Morris J et al. Improved gene transfer into baboon marrow repopulating cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor. Blood. 1998-09-15 [PMID: 9731044] (FLOW)

Roberts SB, Ripellino JA, Ingalls KM et al. Non-amyloidogenic cleavage of the beta-amyloid precursor protein by an integral membrane metalloendopeptidase. J Biol Chem. 1994-01-28 [PMID: 8300647] (WB, Human)

Leitner K, Szlauer R, Ellinger I et al. Placental alkaline phosphatase expression at the apical and basal plasma membrane in term villous trophoblasts. J Histochem Cytochem. 2001-09-01 [PMID: 11511684] (IHC-Fr, ICC/IF, Human)

Kesson AM, Fear WR, Williams L et al. HIV infection of placental macrophages: their potential role in vertical transmission. J Leukoc Biol. 1994-09-01 [PMID: 8083596] (IHC-Fr, Human)

More publications at http://www.novusbio.com/NB110-3638



#### **Procedures**

#### Immunohistochemistry-Paraffin Protocol for Alkaline Phosphatase, Placental Antibody (8B6) (NB110-3638)

Antigen Unmasking - Proteolytic Induced Epitope Retrieval (PIER):

Trypsin Working Solution (0.05%):

Trypsin stock solution (0.5%) -1 ml Calcium chloride stock solution 1% - 1 ml Distilled Water - 8 ml Adjust pH to 7.8 with 1N NaOH.

Cover sections with trypsin working solution and incubate for 10-20 minutes at 37 degrees Celsius in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation, and should be determined by user). Allow sections to cool at room temperature for 10 minutes.

Staining:

- 1. Wash sections in deionized water three times for 5 minutes each.
- 2. Wash sections in wash buffer for 5 minutes.

3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.

4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.

5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.

6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.

7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.

8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.

10. Add 100-400 ul DAB substrate to each section and monitor staining closely.

- 11. As soon as the sections develop, immerse slides in deionized water.
- 12. Counterstain sections in hematoxylin.
- 13. Wash sections in deionized water two times for 5 minutes each.
- 14. Dehydrate sections.
- 15. Mount coverslips.





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