

### Anti-PHAP I ANP32A Antibody

Catalog Number: A03625-1

#### **About ANP32A**

Apoptosis is related to many diseases and development. Caspase-9 plays a central role in cell death induced by a variety of apoptosis activators. Cytochrome c, after released from mitochondria, binds to Apaf-1, which forms an apoptosome that in turn binds to and activate procaspase-9. Activated caspase-9 cleaves and activates the effector caspases (caspase-3, -6 and -7), which are responsible for the proteolytic cleavage of many key proteins in apoptosis. The tumor suppressor putative HLA-DR-associated proteins (PHAPs) were recently identified as important regulators of mitochondrion apoptosis. PHAP appears to facilitate apoptosome-medicated caspase-9 activation and to stimulate the mitochondrial apoptotic pathway. PHAP was also shown to oppose both Ras- and Myc-medicated cell transformation.

#### Overview

Product Name	Anti-PHAP I ANP32A Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-PHAP I ANP32A Antibody (Catalog # A03625-1). Tested in ELISA, WB, ICC, IF applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, IF, ICC, WB
Clonality	Polyclonal
Formulation	PHAP I Antibody is supplied in PBS containing 0.02% sodium azide.
Storage Instructions	PHAP I antibody can be stored at 4°C for three months and -20°C, stable for up to one year. Avoid repeated freeze-thaw cycles. Antibodies should not be exposed to prolonged high temperatures.
Host	Rabbit
Uniprot ID	P39687

### **Technical Details**

Immunogen	Anti-PHAP I antibody was raised against a peptide corresponding to 15 amino acids near the carboxy terminus of human PHAP I. The immunogen is located within the last 50 amino acids of PHAP I.
Predicted Reactive Species	Bovine, Mouse, Rat
Cross Reactivity	This polyclonal antibody has no cross-reaction to PHAP I2a and PHAP III.
Isotype	IgG
Form	Liquid
Concentration	1 mg/mL



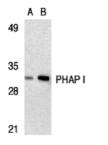


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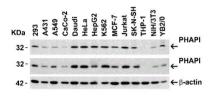
Purification	PHAP I Antibody is DEAE purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.  If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Some PubMed article(s) citing the expression level of this target are as follows:  Boster Bio's internal QC testing used:  WB: 2-4 ug/mL; ICC: 2 ug/mL; IF: 10 ug/mL.  Antibody validated: Western Blot in human, mouse and rat samples; Immunocytochemistry in human samples; Immunofluorescence in human samples. All other applications and species not yet tested. Optimal dilutions for each application should be determined by the researcher.



### Anti-PHAP I ANP32A Antibody (A03625-1) Images

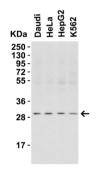


Western Blot Validation in Human Raji Cell Lysate Loading: 15 ug of lysates per lane. Antibodies: PHAP I A03625-1 (A: 2 ug/mL, B: 4 ug/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



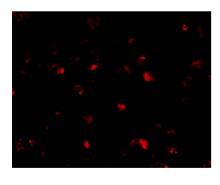
# Independent Antibody Validation (IAV) via Protein Expression Profile in Cell Lines

Loading: 15 ug of lysates per lane.Antibodies: PHAP I A03625-1 (2 ug/mL), PHAP I (1 ug/mL), and beta-actin (1 ug/mL), 1h incubation at RT in 5% NFDM/TBST.Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



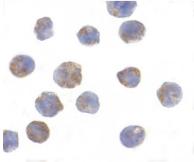
#### **Western Blot Validation in Human Cell Lines**

Loading: 15 ug of lysates per lane. Antibodies: PHAP I A03625-1 (2 ug/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



#### Immunofluorescence Validation of PHAP I in Raii Cells

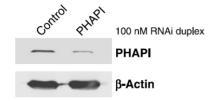
Immunofluorescent analysis of 4% paraformaldehyde-fixed Raji Cells labeling PHAP I with 3145 at 10 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red).

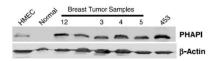


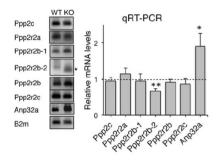
### Immunocytochemistry Validation of PHAP I in Raji

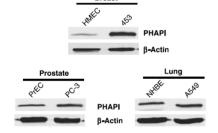
Immunocytochemical analysis of Raji cells using anti-PHAP I antibody (A03625-1) at 2 ug/ml. Cells was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.











### KD Validation of PHAPI in Human Breast Cancer Cells (Schafer et al.)

Human Breast Cancer Cells (T47D cells) were transfected with control or PHAPI siRNA duplex. PHAPI was detected via Western Blot analysis by using the anti-PHAPI antibody. PHAPI expression was reduced after PHAPI siRNA knockdown.

# Increased Expression Validation of PHAPI in Patient Samples of BreastTumor Tissue (Schafer et al.)

PHAPI was overexpressed in all breast tumor samples of patients and human breast cancer cells (MDA-MB-453), but not in the normal breast tissue or human primary mammary epithelial cells (HMEC).

## Induced Expression Validation of PHAPI/Anp32a in Atxn1 KO Mice (Sa´nchez et al., 2013)

Western blot analysis of PHAPI/Anp32a from the cerebellum of WT and Atxn1 KO mice. PHAPI expression was significantly increased (2 folds) in Atxn1 KO mice as compared to WT mice. The same effect was observed in PHAPI mRNA levels.

## Overexpression of PHAPI in Breast Cancer Cells (Schafer et al.)

Western blot analysis with anti-PHAPI antibodies was performed for PHAPI in human cell lines from breast, prostate and lung. PHAPI was overexpressed in breast cancer cells when compared with normal cells (HMEC) whereas there were no significant differences in PHAPI expression in normal and cancer cells of either prostate or lung origin.

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