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antibody and ELISA experts

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Anti-PUMA BBC3 Antibody

Catalog Number: A04899-1

About BBC3

Apoptosis is related to many diseases and development. The p53 tumor-suppressor protein induces apoptosis through transcriptional activation of several genes. A novel p53 inducible pro-apoptotic gene was identified recently and designated PUMA (for p53 upregulated modulator of apoptosis) and bbc3 (for Bcl-2 binding component 3) in human and mouse. PUMA/bbc3 is one of the pro-apoptotic Bcl-2 family members including Bax and Noxa, which are also transcriptional targets of p53 (1). The PUMA gene encodes two BH3 domain-containing proteins termed PUMA-alpha and PUMA-beta (2). PUMA proteins bind Bcl-2, localize to the mitochondria, and induce cytochrome c release and apoptosis in response to p53. PUMA may be a direct mediator of p53-induced apoptosis.

Overview

Product Name	Anti-PUMA BBC3 Antibody
Reactive Species	Human, Rat
Description	Boster Bio Anti-PUMA BBC3 Antibody (Catalog # A04899-1). Tested in ELISA, WB, IHC-P, IF applications. This antibody reacts with Human, Rat.
Application	ELISA, IF, IHC-P, WB
Clonality	Polyclonal
Formulation	PUMA Antibody is supplied in PBS containing 0.02% sodium azide.
Storage Instructions	PUMA 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	Q96PG8

Technical Details

Immunogen	Anti-PUMA antibody was raised against a peptide corresponding to 14 amino acids near the amino terminus of human PUMA isoform 1. The immunogen is located within the first 50 amino acids of PUMA.
Predicted Reactive Species	Mouse
Isotype	IgG
Form	Liquid
Concentration	1 mg/mL
Purification	PUMA Antibody is affinity chromatography purified via peptide column.

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Suggested Dilutions	WB: 2-3 ug/mL; IF: 10-20 ug/mL; IHC: 2.5-10 ug/mL. Antibody validated: Western Blot in human samples; Immunohistochemistry 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.

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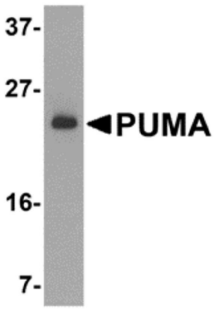
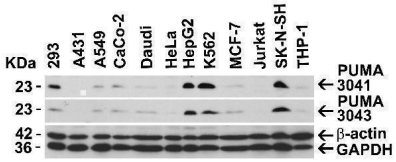
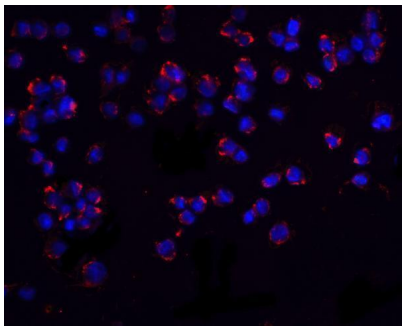
Anti-PUMA BBC3 Antibody (A04899-1) Images



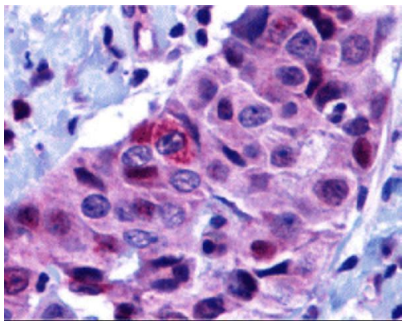
Western Blot Validation of PUMA in K562 Cells Loading: 15 ug of lysates per lane. Antibodies: A04899-1 (2 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution



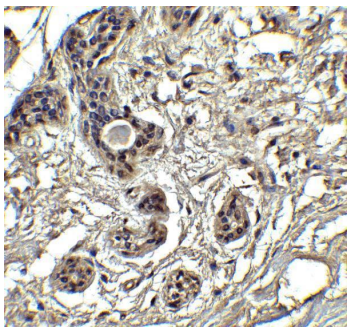
Independent Antibody Validation (IAV) via Protein Expression Profile in Human Cells Loading: 20 ug of lysates per lane. Antibodies: 3041 (3 ug/mL), A04899-1 (2 ug/mL), beta-actin (1 ug/mL) and GAPDH (0.02 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.



Immunofluorescence Validation of PUMA in K562 Cells Immunofluorescent analysis of 4% paraformaldehyde-fixed K562 cells labeling PUMA with A04899-1 at 20 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red) and DAPI staining (blue).



Immunohistochemistry Validation of PUMA in Human Breast Carcinoma Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-PUMA antibody (A04899-1) at 10 ug/ml. Tissue 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.



Immunohistochemistry Validation of PUMA in Human Breast Tissue Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-PUMA antibody (A04899-1) at 2.5 ug/ml. Tissue 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.

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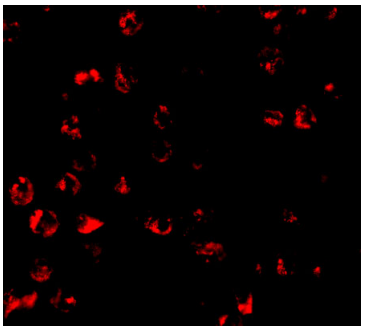
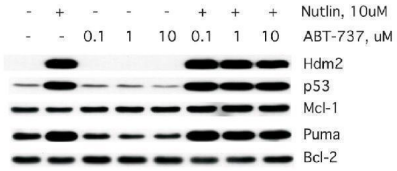
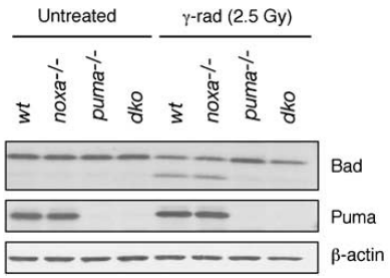


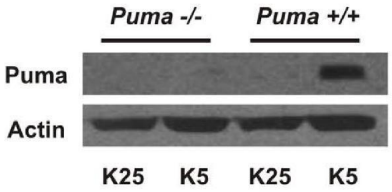
Immunofluorescence Validation of PUMA in K562
Immunofluorescent analysis of 4% paraformaldehyde-fixed K562 cells labeling PUMA with A04899-1 at 10 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red). Image showing cytosol staining on K562 cells



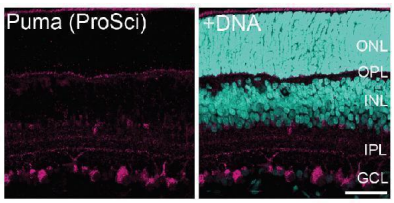
Induced Expression of PUMA in MCF7 cells (Wade et al., 2008) Western analysis of MCF7 treated with the indicated dose of Nutlin-3a or ABT-737 for 24h. Note that Puma is induced following Nutlin-3a treatment in these cells and PUMA expression was detected by anti-PUMA antibodies (A04899-1)



KO Validation of PUMA in Mouse Thymocytes (Michalak et al., 2008) Western blot analysis of thymocytes from wt, noxa knockout, puma knockout and noxa/puma double knockout mice cultured for 7 h in the presence or absence of 2.5 Gy g-irradiation. PUMA expression was not detected in puma KO and double KO mice with anti-PUMA antibodies (A04899-1).



KO Validation of PUMA in Mouse Cerebellar Neurons (Ambacher et al., 2012) Puma expression is induced by potassium withdrawal in cerebellar granule neurons. After 7 days in culture CGNs were either maintained in media containing 25 mM potassium (K25) or switched to low potassium medium containing 5 mM potassium (K5). PUMA protein levels were analyzed by western blot with anti-PUMA antibodies (A04899-1). PUMA expression was not detected in PUMA KO mice and was increased after treatment in WT.



Immunofluorescence Validation of PUMA in Rat Retina (Wakabayashi et al., 2012) PUMA expression in the rat retina detected by anti-PUMA antibodies (A04899-1). The specimens were counterstained with Hoechst 33258 to visualize nuclei (+DNA). GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; P, postnatal day.

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