

Product Datasheet

AlphaB Crystallin/CRYAB Antibody (OTI6A9) NBP1-47708

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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NBP1-47708

AlphaB Crystallin/CRYAB Antibody (OTI6A9)

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	OTI6A9
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Immunogen affinity purified
Buffer	PBS (pH 7.3), 1.0% BSA and 50% Glycerol
Target Molecular Weight	20 kDa

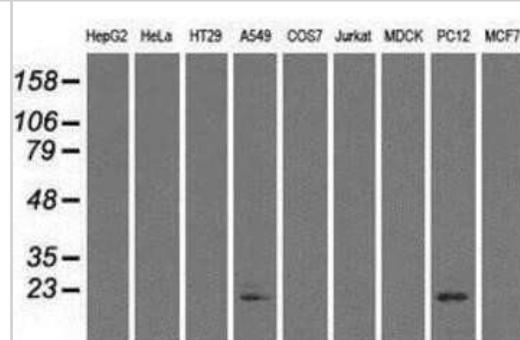
Product Description	
Description	Novus Biologicals Mouse AlphaB Crystallin/CRYAB Antibody (OTI6A9) (NBP1-47708) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-AlphaB Crystallin/CRYAB Antibody: Cited in 1 publication. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	1410
Gene Symbol	CRYAB
Species	Human, Mouse, Rat
Reactivity Notes	Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Mouse-On-Mouse blocking reagent may be needed for IHC and ICC experiments to reduce high background signal. You can find these reagents under catalog numbers PK-2200-NB and MP-2400-NB. Please contact Technical Support if you have any questions.
Specificity/Sensitivity	This antibody is specific for Homo sapiens crystallin, alpha B (CRYAB).
Immunogen	Full length human recombinant protein of human CRYAB (NP_001876) produced in HEK293T cell.

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500, Flow Cytometry 1:100, Immunohistochemistry 1:50, Immunocytochemistry/ Immunofluorescence 1:50-100, Immunoprecipitation 2ug/500ul, Immunohistochemistry-Paraffin 1:50

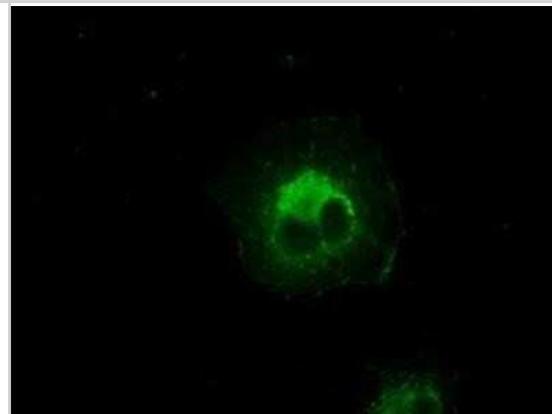


Images

Western Blot: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - Analysis of extracts (35ug) from 9 different cell lines by using anti-Crystallin AB monoclonal antibody (HepG2: human; HeLa: human; SVT2: mouse; A549: human; COS7: monkey; Jurkat: human; MDCK: canine; PC12: rat; MCF7: human).



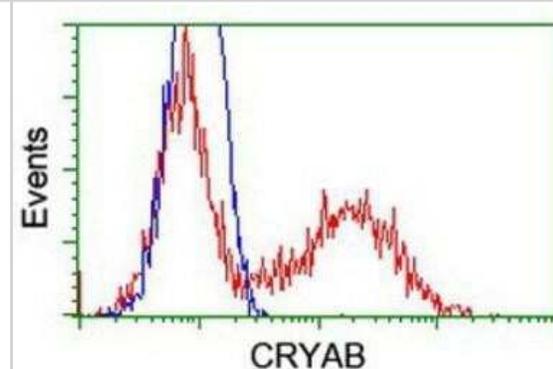
Immunocytochemistry/Immunofluorescence: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - Staining of COS7 cells transiently transfected by pCMV6-ENTRY Crystallin AB.



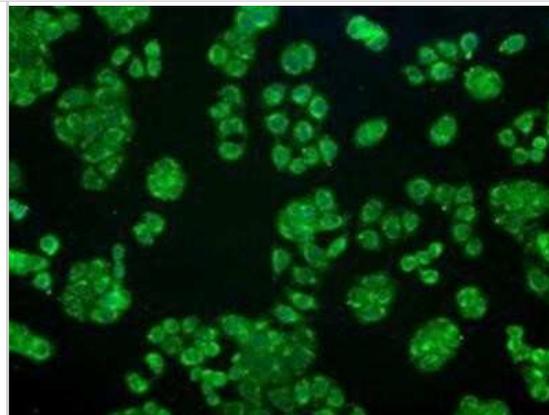
Immunohistochemistry-Paraffin: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - Staining of paraffin-embedded Human pancreas tissue using anti-Crystallin AB mouse monoclonal antibody.



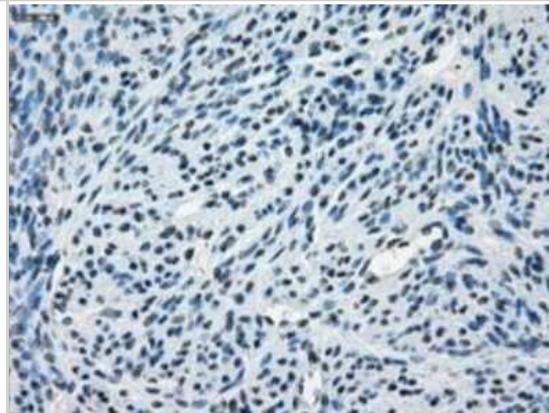
Flow Cytometry: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - HEK293T cells transfected with either overexpression plasmid (Red) or empty vector control plasmid (Blue) were immunostained by anti-Crystallin AB antibody, and then analyzed by flow cytometry.



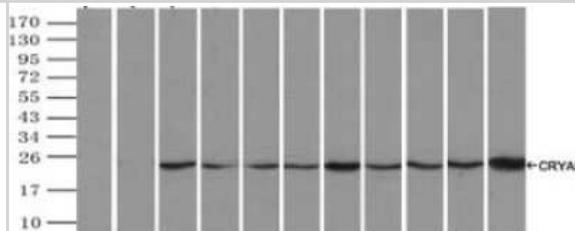
Immunocytochemistry/Immunofluorescence: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - Staining of HT29 cells using anti-Crystallin AB mouse monoclonal antibody.



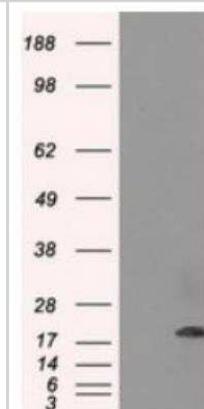
Immunohistochemistry-Paraffin: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - Staining of paraffin-embedded Human endometrium tissue using anti-Crystallin AB mouse monoclonal antibody.



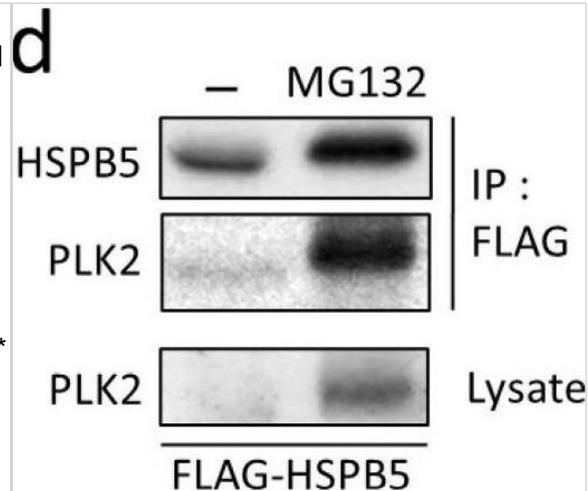
Immunoprecipitation: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - (Negative control: IP without adding anti-CRYAB antibody.). For each experiment, 500ul of DDK tagged CRYAB overexpression lysates (at 1:5 dilution with HEK293T lysate), 2ug of anti-CRYAB antibody and 20ul (0.1mg) of goat anti-mouse conjugated magnetic beads were mixed and incubated overnight. After extensive wash to remove any non-specific binding, the immuno-precipitated products were analyzed with rabbit anti-DDK polyclonal antibody.



Western Blot: AlphaB Crystallin/CRYAB Antibody (OTI6A9) [NBP1-47708] - HEK293T cells were transfected with the pCMV6-ENTRY control (Left lane) or pCMV6-ENTRY CRYAB (Right lane) cDNA for 48 hrs and lysed. Equivalent amounts of cell lysates (5 ug per lane) were separated by SDS-PAGE and immunoblotted with AlphaB Crystallin/CRYAB Antibody (OTI6A9).

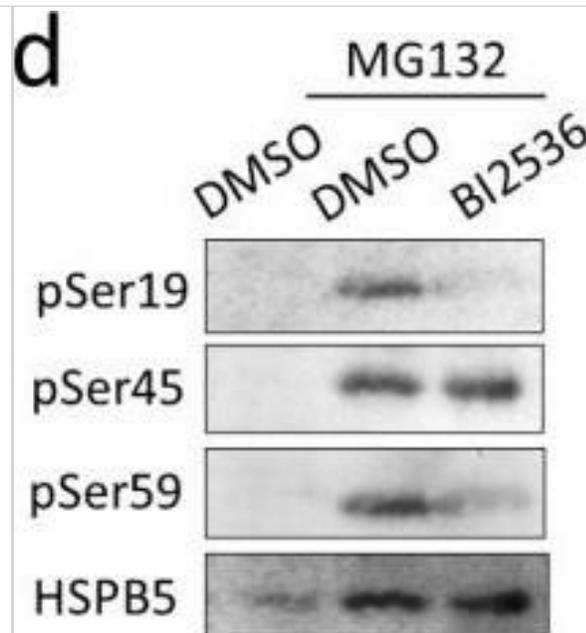


PLK2, a novel binding protein of HSPB5, is induced by MG132 in L6 cells. (a) Schematic diagram of domain structure of PLK2. K90, K95, and K111 indicate position of lysine residue (K) biotinylated by HSPB5-BioID2. Lysine residues were numbered based on amino acid sequence of PLK2 derived from Homo sapiens (NCBI NP_006613). (b) Comparison of induction of HSPB5 and PLK2 expression by ER stress-induced drugs. Differentiated L6 cells were treated with 5 μ M MG132 or 0.1 μ g/mL tunicamycin for 24 h. Induction of HSPB5, and PLK2 protein expression was detected by Western blotting. (c) Graph shows levels of endogenous proteins induced by the drug. Data represent means \pm standard error (n = 3). Statistical analyses were performed using t-test. ** p < 0.01; n.s. means not significant. (d) Verification of binding of PLK2 to HSPB5. FLAG-HSPB5 was overexpressed in L6 cells by liposome transfection. After 5 μ M MG132 treatment (24 h), cells were solubilized, and HSPB5 pull-down assay was performed using an anti-tag antibody. (e) Quantification of binding of PLK2 to HSPB5. Control cells were transfected with empty vector. After 5 μ M MG132 treatment (24 h), a pull-down assay was performed using an anti-tag antibody. Graph presents level of endogenous PLK2 pulled down by HSPB5. Data represent means \pm standard error (n = 3). Statistical analyses were performed using t-test. * p < 0.05. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36232565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

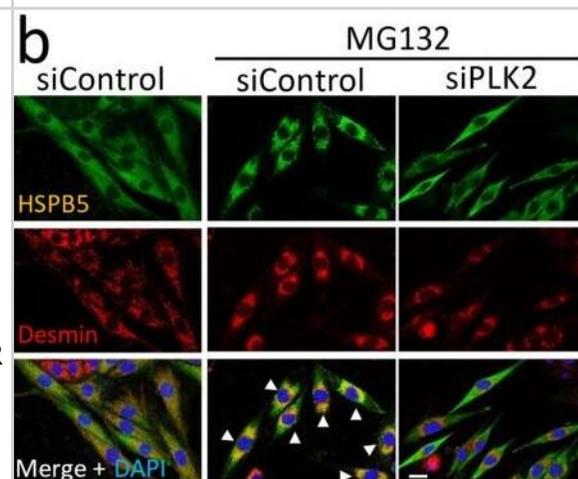


PLK2, a novel binding protein of HSPB5, is induced by MG132 in L6 cells. (a) Schematic diagram of domain structure of PLK2. K90, K95, and K111 indicate position of lysine residue (K) biotinylated by HSPB5-BioID2. Lysine residues were numbered based on amino acid sequence of PLK2 derived from Homo sapiens (NCBI NP_006613). (b) Comparison of induction of HSPB5 and PLK2 expression by ER stress-induced drugs. Differentiated L6 cells were treated with 5 μ M MG132 or 0.1 μ g/mL tunicamycin for 24 h. Induction of HSPB5, and PLK2 protein expression was detected by Western blotting. (c) Graph shows levels of endogenous proteins induced by the drug. Data represent means \pm standard error (n = 3). Statistical analyses were performed using t-test. ** p < 0.01; n.s. means not significant. (d) Verification of binding of PLK2 to HSPB5. FLAG-HSPB5 was overexpressed in L6 cells by liposome transfection. After 5 μ M MG132 treatment (24 h), cells were solubilized, and HSPB5 pull-down assay was performed using an anti-tag antibody. (e) Quantification of binding of PLK2 to HSPB5. Control cells were transfected with empty vector. After 5 μ M MG132 treatment (24 h), a pull-down assay was performed using an anti-tag antibody. Graph presents level of endogenous PLK2 pulled down by HSPB5. Data represent means \pm standard error (n = 3). Statistical analyses were performed using t-test. * p < 0.05. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36232565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

PLK2 phosphorylates HSPB5 at serine 19 under ER stress. (a) Knockdown efficiency of PLK2 by siRNA. L6 cells were transfected with siRNA to knockdown PLK2 and treated with 5 μ M MG132 for 24 h. Graph presents relative value of expression level of PLK2. Data in graphs represent means \pm standard error ($n = 3$). Statistical analyses were performed using t-test. ** $p < 0.01$. (b) Schematic representation of three known phosphorylation sites of HSPB5. Arrows indicate name of the kinase catalyzing phosphorylation. (c) Effect of PLK2 on each phosphorylation site. Undifferentiated L6 cells were transfected with siRNA to knockdown PLK2. After differentiation (48 h), L6 myotubes were treated with 5 μ M MG132 for 24 h. Phosphorylation level was detected by Western blotting with specific antibodies. Graph presents ratio of phosphorylated HSPB5/HSPB5 after quantification of each band. Data in graphs represent means \pm standard error ($n = 3$). Statistical analyses were performed using one-way ANOVA with Tukey test. ‡ $p < 0.01$, † $p < 0.05$; n.s. not significant. (d) Effect of PLK2 activity on phosphorylation of serine 19. After differentiation (48 h), L6 myotubes were treated with 10 nM BI2536 and 5 μ M MG132 for 24 h. Phosphorylation level was detected by Western blotting with specific antibodies. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36232565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Relationship between phosphorylation and subcellular localization of HSPB5. (a) Localization of overexpressed recombinant HSPB5 and endogenous PLK2. After 5 μ M MG132 treatment (24 h), FLAG-tagged HSPB5 and endogenous PLK2 in undifferentiated L6 cells were immunofluorescently stained with anti-tag and anti-PLK2 antibodies. (b) Effect of PLK2 on localization of HSPB5 and desmin protein. Endogenous HSPB5 and desmin were immunofluorescently stained with anti-HSPB5 and anti-desmin antibodies. Undifferentiated L6 cells were transfected with siRNA to knockdown PLK2. After differentiation (48 h), L6 myotubes were treated with MG132 for 24 h. White arrowheads indicate cells with colocalization of HSPB5 and desmin at perinuclear ER region. Scale bar indicates 20 μ m. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36232565>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Ueda S, Nishihara M, Hioka Y et al. Polo-Like Kinase 2 Plays an Essential Role in Cytoprotection against MG132-Induced Proteasome Inhibition via Phosphorylation of Serine 19 in HSPB5 International Journal of Molecular Sciences 2022-09-24 [PMID: 36232565] (Immunocytochemistry/ Immunofluorescence)



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NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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