

Product Datasheet

PKN3 Antibody - BSA Free

NBP1-30102

Unit Size: 100 ul

Store at 4C. Do not freeze.

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NBP1-30102

PKN3 Antibody - BSA Free

Product Information

Unit Size	100 ul
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)
Target Molecular Weight	99 kDa

Product Description

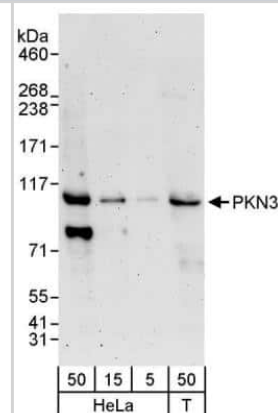
Description	Novus Biologicals Knockout (KO) Validated Rabbit PKN3 Antibody - BSA Free (NBP1-30102) is a polyclonal antibody validated for use in WB. Anti-PKN3 Antibody: Cited in 6 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	29941
Gene Symbol	PKN3
Species	Human, Mouse
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 30422386).
Immunogen	maps to a region between residue 225 and 275 of human protein kinase N3 using the numbering given in entry NP_037487.2

Product Application Details

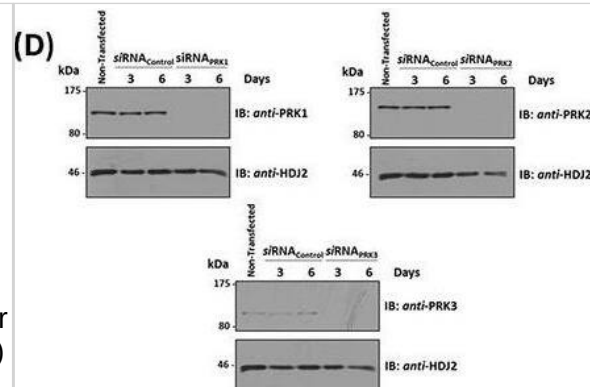
Applications	Western Blot, Immunoprecipitation (Negative), Knockout Validated
Recommended Dilutions	Western Blot 1:2000-1:10000, Immunoprecipitation (Negative), Knockout Validated Reactivity validation reported in (PMID: 30518210)

Images

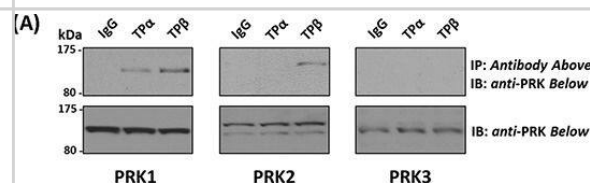
Western Blot: PKN3 Antibody [NBP1-30102] - Whole cell lysate from HeLa (5, 15 and 50 ug) and 293T (T; 50 ug) cells. Antibody used at 0.1 ug/ml.



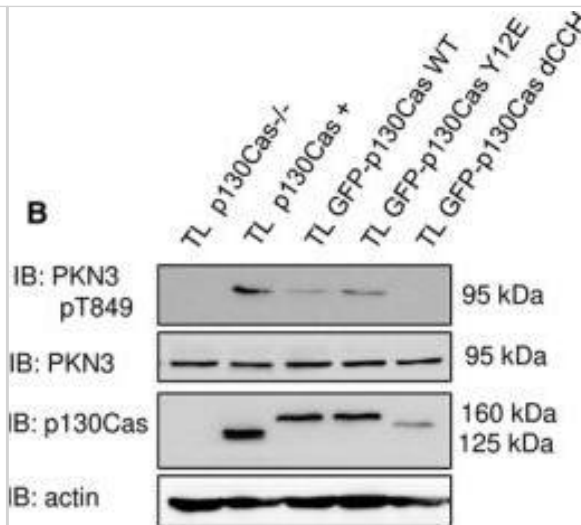
Western Blot: PKN3 Antibody [NBP1-30102] - Effect of siRNA-mediated disruption of PRK1, PRK2 & PRK3 expression on TP agonist-induced proliferation & migration of PC-3 cells Panels A-D. PC-3 cells were transfected for 48 hr with 30 nM siRNAPRK1, siRNAPRK2, siRNAPRK3 or, as controls, with a scrambled siRNA (siRNAControl) prior to stimulation with U46619 (10 nM) or vehicle (0.0001% EtOH) for the indicated time specific to the assay, where non-transfected cells served as a reference. Panel A: For analysis of proliferation, 72 hr post-siRNA transfection PC-3 cells were stimulated for 48 hr with U46619 (10 nM) or vehicle (0.0001% EtOH). Panel B: For analysis of colony formation, 48 hr post-siRNA transfection PC-3 cells were stimulated with U46619 (10 nM) or vehicle (0.0001% EtOH) in soft agar & assessed 10 day post-treatment for colony formation. Panel C: For analysis of migration, 72 hr post-siRNA transfection PC-3 cells were stimulated for 8 hr with U46619 (10 nM) or vehicle (0.0001% EtOH). In Panels A-C, the bar charts show mean relative levels of PC-3 cell proliferation, colony formation & migration ($\pm$ SEM, $n \geq 3$), where levels in the vehicle-treated cells are assigned a value of 100%. The asterisks indicate where U46619-stimulation resulted in significant increases in proliferation, colony formation or migration by PC-3 cells compared to vehicle-treated cells, where ** & *** indicates $p < 0.01$ & $p < 0.001$, respectively. Panel D: Validation of the specificity & sustained siRNA-mediated disruption of PRK1, PRK2 & PRK3 expression was confirmed by immunoblot analysis of whole cell lysates (20 μ g/lane) with the respective anti-PRK1/2/3 or, to validate protein loading, with anti-HDJ2 antisera where analysis was carried out 3 & 6 day post-transfection. Data $n \geq 3$. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.4664>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



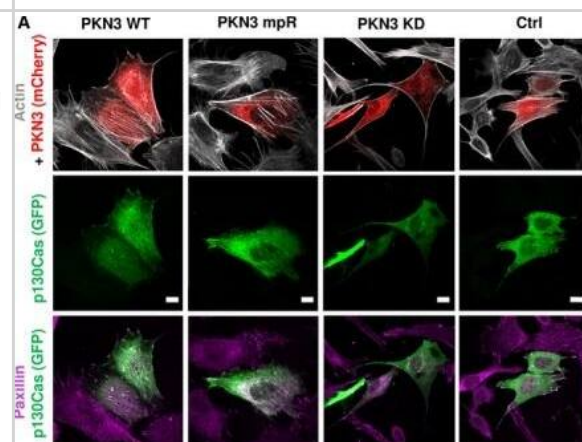
Western Blot: PKN3 Antibody [NBP1-30102] - Association of PRK1 & PRK2 with TP α & TP β in prostate PC-3 cells Panel A. PC-3 cells were immunoprecipitated with anti-TP α , anti-TP β or, as controls, with the pre-immune (IgG) sera. Thereafter, immunoprecipitates (upper panels) or equivalent aliquots of whole cell lysates (20 μ g/lane, lower panels) were immunoblotted (IB) with anti-PRK1, anti-PRK2 or anti-PRK3 antisera. The relative positions of the molecular size markers (kDa) are indicated to the left of the panels. Data shown are representative of at least three independent experiments ($n \geq 3$). Panel B. PC-3 cells were incubated with U46619 (1 μ M; 0–60 min) prior to immunoprecipitation with anti-TP α , anti-TP β or, as controls, with the pre-immune (IgG) sera. Thereafter, immunoprecipitates (upper panels) or equivalent aliquots of whole cell lysates (20 μ g/lane, lower panels) were IB with anti-PRK1, anti-PRK2 or anti-PRK3 antisera. Data $n \geq 3$. Panel C. Bar charts show the mean relative levels of PRK1 or PRK2 associated with the anti-TP α or anti-TP β immunoprecipitates, as determined by quantitative densitometry ($\pm$ SEM), where levels associated with the respective immunoprecipitates in the absence of agonist are expressed as 1. The asterisks indicate where U46619 stimulation resulted in significant changes in complex-associated PRK1 or PRK2, where * & ** indicate $p < 0.05$ & $p < 0.01$, respectively. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.4664>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: PKN3 Antibody [NBP1-30102] - PKN3 activity is important for stress fibers formation & is stimulated by the expression of p130Cas. (A) p130Cas^{-/-}-MEFs growing on FN-coated cover slips were co-transfected by GFP-p130Cas & mCherry-PKN3 fusion variant (WT, mPR, KD) or mCherry. After 48 h, cells were fixed & imaged by Leica TCS SP2 microscope (63×/1.45 oil objective). Stress fibers were visualized by Phalloidin (405) & focal adhesions by anti-Paxillin staining (2nd 633). Representative images are shown. Scale bars represent 20 μm. (B) p130Cas^{-/-}-MEFs or p130Cas^{-/-}-MEFs re-expressing p130Cas or transfected by GFP-fused p130Cas variants (WT, YE, dCCH) were lysed in RIPA buffer, blotted to nitrocellulose membrane, & analyzed for endogenous PKN3 activity by antibody anti-phosphoThr849 of PKN3 (pT849 PKN3). Expression of p130Cas mutants was verified by anti-p130Cas antibody & loading by anti-PKN3 & anti-actin antibody. (C) Densitometric quantification of PKN3 activity (pT849 PKN3 phosphorylation). The effect of p130Cas re-expression on PKN3 T849 phosphorylation was analyzed separately from the effect of transfected p130Cas mutants (indicated by a dotted line). Error bars indicate means ± SD from three independent experiments (four experiments for the left part). Statistical significance was evaluated by one-way repeated ANOVA followed by Turkey's post hoc test (*P < 0.05; **P < 0.01). (D) Lysates or (E) immunoprecipitates (by Flag sepharose) from p130Cas^{-/-}-MEFs re-expressing p130Cas & overexpressing PKN3 variants (WT, mPR, KD) were immunoblotted by anti-PKN3, anti-pT849 PKN3, & anti-Akt antibodies (loading control). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30422386>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



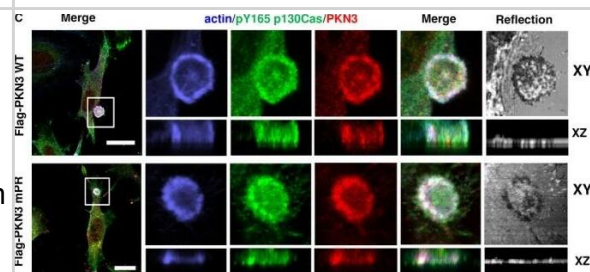
Immunocytochemistry/ Immunofluorescence: PKN3 Antibody [NBP1-30102] - PKN3 activity is important for stress fibers formation & is stimulated by the expression of p130Cas. (A) p130Cas^{-/-}-MEFs growing on FN-coated cover slips were co-transfected by GFP-p130Cas & mCherry-PKN3 fusion variant (WT, mPR, KD) or mCherry. After 48 h, cells were fixed & imaged by Leica TCS SP2 microscope (63×/1.45 oil objective). Stress fibers were visualized by Phalloidin (405) & focal adhesions by anti-Paxillin staining (2nd 633). Representative images are shown. Scale bars represent 20 μm. (B) p130Cas^{-/-}-MEFs or p130Cas^{-/-}-MEFs re-expressing p130Cas or transfected by GFP-fused p130Cas variants (WT, YE, dCCH) were lysed in RIPA buffer, blotted to nitrocellulose membrane, & analyzed for endogenous PKN3 activity by antibody anti-phosphoThr849 of PKN3 (pT849 PKN3). Expression of p130Cas mutants was verified by anti-p130Cas antibody & loading by anti-PKN3 & anti-actin antibody. (C) Densitometric quantification of PKN3 activity (pT849 PKN3 phosphorylation). The effect of p130Cas re-expression on PKN3 T849 phosphorylation was analyzed separately from the effect of transfected p130Cas mutants (indicated by a dotted line). Error bars indicate means ± SD from three independent experiments (four experiments for the left part). Statistical significance was evaluated by one-way repeated ANOVA followed by Turkey's post hoc test (*P < 0.05; **P < 0.01). (D) Lysates or (E) immunoprecipitates (by Flag sepharose) from p130Cas^{-/-}-MEFs re-expressing p130Cas & overexpressing PKN3 variants (WT, mPR, KD) were immunoblotted by anti-PKN3, anti-pT849 PKN3, & anti-Akt antibodies (loading control). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30422386>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



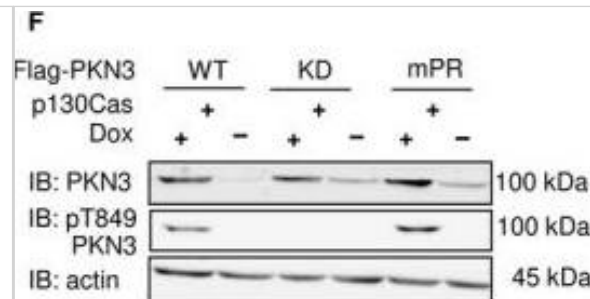
Western Blot: PKN3 Antibody [NBP1-30102] - PKN3 activity is important for stress fibers formation & is stimulated by the expression of p130Cas. (A) p130Cas^{-/-}-MEFs growing on FN-coated cover slips were co-transfected by GFP-p130Cas & mCherry-PKN3 fusion variant (WT, mPR, KD) or mCherry. After 48 h, cells were fixed & imaged by Leica TCS SP2 microscope (63×/1.45 oil objective). Stress fibers were visualized by Phalloidin (405) & focal adhesions by anti-Paxillin staining (2nd 633). Representative images are shown. Scale bars represent 20 μ m. (B) p130Cas^{-/-}-MEFs or p130Cas^{-/-}-MEFs re-expressing p130Cas or transfected by GFP-fused p130Cas variants (WT, YE, dCCH) were lysed in RIPA buffer, blotted to nitrocellulose membrane, & analyzed for endogenous PKN3 activity by antibody anti-phosphoThr849 of PKN3 (pT849 PKN3). Expression of p130Cas mutants was verified by anti-p130Cas antibody & loading by anti-PKN3 & anti-actin antibody. (C) Densitometric quantification of PKN3 activity (pT849 PKN3 phosphorylation). The effect of p130Cas re-expression on PKN3 T849 phosphorylation was analyzed separately from the effect of transfected p130Cas mutants (indicated by a dotted line). Error bars indicate means $\pm$ SD from three independent experiments (four experiments for the left part). Statistical significance was evaluated by one-way repeated ANOVA followed by Turkey's post hoc test (* $P < 0.05$; ** $P < 0.01$). (D) Lysates or (E) immunoprecipitates (by Flag sepharose) from p130Cas^{-/-}-MEFs re-expressing p130Cas & overexpressing PKN3 variants (WT, mPR, KD) were immunoblotted by anti-PKN3, anti-pT849 PKN3, & anti-Akt antibodies (loading control). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30422386>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: PKN3 Antibody [NBP1-30102] - PKN3 colocalizes with p130Cas in lamellipodia & podosome rosettes. Representative images are shown. (A) p130Cas^{-/-}-MEFs plated on fibronectin (FN) were transfected by GFP-p130Cas, CFP-LifeAct, & mCherry-PKN3WT or mCherry-PKN3 mPR & imaged live 24 h after transfection. White arrow indicates lamellipodia. Histogram of dotted straight line is shown. (B) Quantification of mCherry-PKN3 WT, mCherry-PKN3 mPR, & mCherry localization to lamellipodia (LifeAct as marker) was calculated as described in methods (values are mean $\pm$ SD from three independent experiments, $n > 50$ measurements – 3 per cell; *** $P < 0.001$, one-way ANOVA on ranks followed by Dunn's post hoc test). (C) Src-transformed p130Cas^{-/-}-MEFs co-expressing p130Cas (SC) & mouse Flag tagged PKN3 WT or Flag-PKN3 mPR are shown. Cells were grown on FN-coated coverslips for 48 h, fixed, & stained for p130Cas by anti-pTyr165 p130Cas antibody (pY165 p130Cas; 2nd 405), for actin by Phalloidin 488 & for Flag-PKN3 by anti-Flag antibody (2nd 633). Reflection (670 nm) indicates fibronectin degradation. All scale bars represent 20 μ m. Cell were imaged by Leica TCS SP8 microscope system equipped with Leica 63×/1.45 oil objective. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30422386>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: PKN3 Antibody [NBP1-30102] - PKN3 overexpression regulates growth of MEFs, & this effect requires PKN3–p130Cas interaction. (A) Immunoblotted lysates from MEFs p130Cas^{-/-} or MEFs p130Cas^{-/-} re⁺expressing p130Cas (p130Cas⁺) treated by Doxycycline (Dox) to induce expression of mCherry⁺PKN3 or mCherry alone. p130Cas presence detected by anti⁺p130Cas antibody & mCherry epitope by anti⁺mCherry antibody. (B) Dynamics of mCherry⁺PKN3 expression after supplementation w/ Dox shown by immunoblot w/ anti⁺mCherry antibody. (C–E) Effect of induced mCherry⁺PKN3 expression on cell growth. Representative graphs showing growth of MEFs p130Cas^{-/-} re⁺expressing p130Cas (p130Cas⁺) (C) or MEFs p130Cas^{-/-} (D) measured in real⁺time using xCELLigence RTCA (real⁺time cell analysis) system instrument. (E) Quantification of cell growth change induced by mCherry⁺PKN3 expression ('-' indicates inducible mCherry expression used as negative control). Slope ratios reflecting cell growth calculated from log growth phase of cell growth (indicated by dotted lines; see C & D). (F) Immunoblotted lysates from MEFs p130Cas^{-/-} re⁺expressing p130Cas (p130Cas⁺) treated or not treated by Dox which induced expression of Flag⁺fused PKN3 variants (WT, mPR, KD, empty vector). Stimulated overexpression of PKN3 detected by anti⁺PKN3 antibody & its activity by antibody anti⁺pT849 PKN3. (G) Quantification of cell growth change stimulated by Dox⁺inducible expression of Flag⁺fused PKN3 variants (WT, mPR, KD) in MEFs p130Cas^{-/-} re⁺expressing p130Cas (p130Cas⁺). All error bars indicate means $\pm$ SD calculated from 3 to 5 independent experiments (each in triplicates). Statistical significance always calculated between induced & noninduced cells & evaluated by one⁺way repeated ANOVA followed by Turkey post hoc test (**P < 0.001). Image collected & cropped by CiteAb from following publication (<https://pubmed.ncbi.nlm.nih.gov/30422386>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Aine G O'Sullivan, Eamon P Mulvaney, B Therese Kinsella Regulation of protein kinase C-related kinase (PRK) signalling by the TP α and TP β isoforms of the human thromboxane A 2 receptor: Implications for thromboxane- and androgen- dependent neoplastic and epigenetic responses in prostate cancer. *Biochimica et biophysica acta. Molecular basis of disease* 2018-11-19 [PMID: 28108419]

Arang N, Lubrano S, Ceribelli M et al. High-throughput chemogenetic drug screening reveals PKC-RhoA/PKN as a targetable signaling vulnerability in GNAQ-driven uveal melanoma Cell reports. *Medicine* 2023-10-13 [PMID: 37858338] (WB, Human)

Browne CM, Jiang B, Ficarro SB et al. A Chemoproteomic Strategy for Direct and Proteome-Wide Covalent Inhibitor Target-Site Identification J. Am. Chem. Soc. 2019-01-09 [PMID: 30518210] (KO, WB, Human)

Gemperle J, Dibus M, Koudelkova L et al. The interaction of p130Cas with PKN3 promotes malignant growth Mol Oncol. [PMID: 30422386] (WB, Mouse)

O'Sullivan AG, Mulvaney EP, Hyland PB, Kinsella BT. Protein kinase C-related kinase 1 and 2 play an essential role in thromboxane-mediated neoplastic responses in prostate cancer. *Oncotarget* 2015-09-22 [PMID: 26296974]

Collazos A, Michael N, Whelan RD et al. Site recognition and substrate screens for PKN family proteins. *Biochem J*;438(3):535-43. 2011-09-15 [PMID: 21749319]



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NBP2-24891	Rabbit IgG Isotype Control
NBP2-33444PEP	PKN3 Recombinant Protein Antigen

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