

# Product Datasheet

## Rictor Antibody - BSA Free

### NB100-612

Unit Size: 100 ul

Store at 4C. Do not freeze.

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**NB100-612**

Rictor Antibody - BSA Free

**Product Information**

<b>Unit Size</b>	100 ul
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C. Do not freeze.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.09% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	Tris-Citrate/Phosphate (pH 7.0 - 8.0)
<b>Target Molecular Weight</b>	192 kDa

**Product Description**

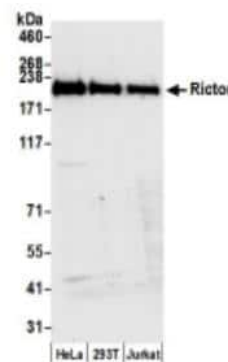
<b>Host</b>	Rabbit
<b>Gene ID</b>	253260
<b>Gene Symbol</b>	RICTOR
<b>Species</b>	Human, Mouse
<b>Immunogen</b>	The immunogen recognized by this antibody maps to a region between residue 1650 and the C-terminus (residue 1708) of human Rapamycin-Insensitive Companion of mTOR using the numbering given in TrEMBL entry Q6R327 (GeneID 253260).

**Product Application Details**

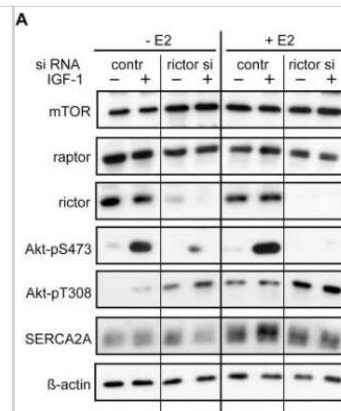
<b>Applications</b>	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot 1:2000-1:10000, Immunohistochemistry 1:500-1:2000, Immunocytochemistry/ Immunofluorescence 1:50 - 1:250, Immunoprecipitation 2-5 ug/mg lysate, Immunohistochemistry-Paraffin 1:500-1:2000, Knockdown Validated
<b>Application Notes</b>	Use in ICC/IF reported in scientific literature (PMID 25378594). Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.

**Images**

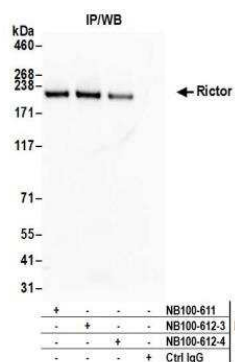
Western Blot: Rictor Antibody [NB100-612] - Whole cell lysate (50 ug) from HeLa, HEK293T, and Jurkat cells prepared using NETN lysis buffer. Antibody: Affinity purified rabbit anti-Rictor antibody used for WB at 0.1 ug/ml. Detection: Chemiluminescence with an exposure time of 30 seconds.



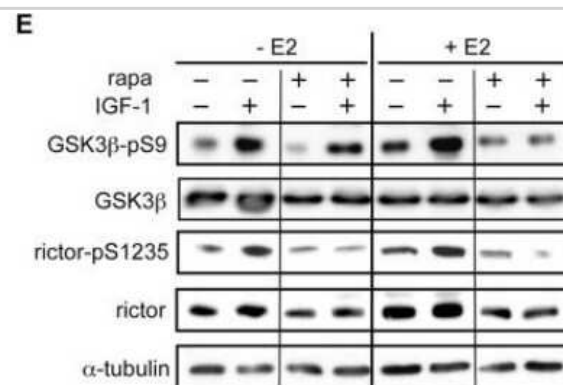
**Western Blot: Rictor Antibody [NB100-612] - SERCA2A expression is regulated by mTORC2.** Rictor silencing was induced by cell transfection with rictor siRNA and then, cells were stimulated with IGF-1 for 24 hours in presence or absence of E2. mTORC2 downregulation was confirmed by abolished Akt-pS473 and resulted in decreased SERCA2A protein expression. Akt-pT308, mTOR and raptor were not negatively affected by rictor silencing. Pictured are representative western blots. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0123385>), licensed under a CC-BY license.



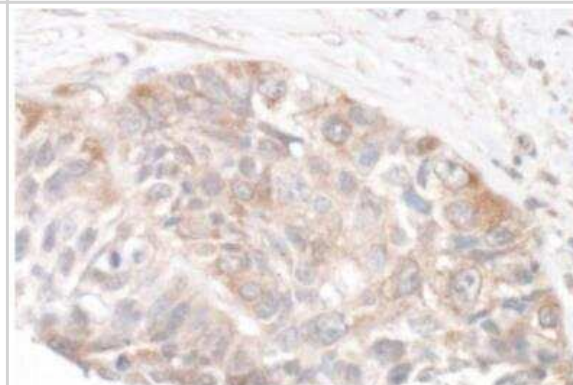
**Immunoprecipitation: Rictor Antibody [NB100-612] - Detection of human Rictor by western blot of immunoprecipitates.** Samples: Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from HeLa cells prepared using NETN lysis buffer. Antibodies: Affinity purified rabbit anti-Rictor antibody NB100-612 (lot NB100-612-4) used for IP at 3 ug per reaction. Rictor was also immunoprecipitated by a previous lot of this antibody (lot NB100-612-3) and rabbit anti-Rictor antibody NB100-611. For blotting immunoprecipitated Rictor, NB100-612 was used at 1 ug/ml. Detection: Chemiluminescence with an exposure time of 10 seconds.



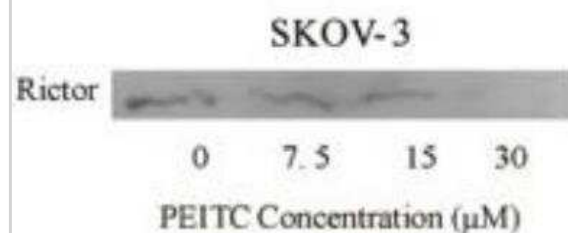
**Western Blot: Rictor Antibody [NB100-612] - mTOR complex protein expressions & rictor phosphorylation at Ser1235.** Cardiomyocytes were pretreated for 30 min with 20 nM rapamycin, then stimulated with 10 nM IGF-1 in presence/absence of 10 nM E2 for 24 h. Western blots for GSK3β-pS9, GSK3β, rictor pS1235 and rictor. At least 3 independent experiments indicate that rictor phosphorylation at S1235 by GSK-3β downregulates mTORC2 activity. IGF-1 induced strong phosphorylation of GSK-3β at Ser9 in the absence and presence of E2. Rapamycin pretreatment reduced phosphorylation in E2 co-treated cardiomyocytes, indicating increased activity of GSK-3β, which was not associated with increased phosphorylation of rictor at S1235. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0123385>), licensed under a CC-BY license.



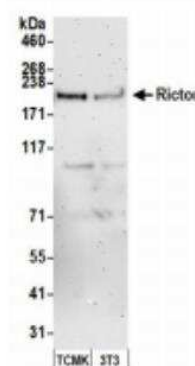
**Immunohistochemistry-Paraffin: Rictor Antibody [NB100-612] - Human lung carcinoma.** Antibody: Affinity purified rabbit anti-Rictor used at a dilution of 1:1,000 (1ug/ml). Detection: DAB



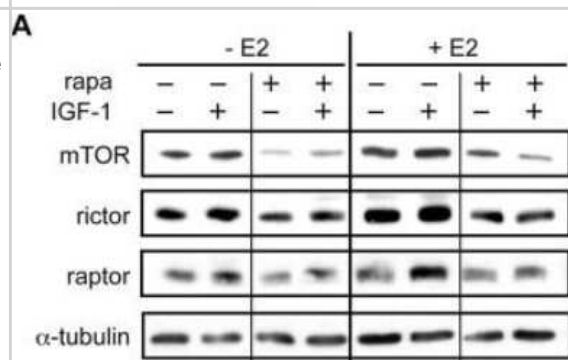
Western Blot: Rictor Antibody [NB100-612] - Detection of RICTOR on SKOV3 whole cell lysate, treated with PEITC. Image courtesy of product review by Parul Gupta (Texas Tech University Health Sciences Center).



Western Blot: Rictor Antibody [NB100-612] - Whole cell lysate (50  $\mu$ g) from TCMK-1 and NIH 3T3 cells prepared using NETN lysis buffer. Antibody: Affinity purified rabbit anti-Rictor antibody used for WB at 0.1  $\mu$ g/ml. Detection: Chemiluminescence with an exposure time of 3 minutes.

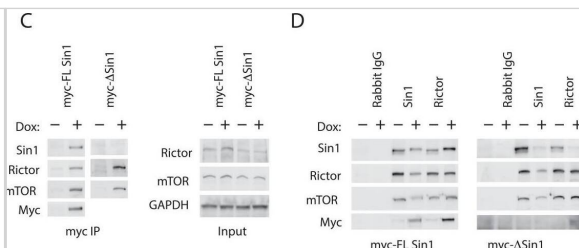


Western Blot: Rictor Antibody [NB100-612] - mTOR complex protein expressions and rictor phosphorylation at Ser1235. Cardiomyocytes were pretreated for 30 min with 20 nM rapamycin and then stimulated with 10 nM IGF-1 in presence or absence of 10 nM E2 for 24 h. A, shown are representative western blots for mTOR, rictor and raptor; quantitative analysis was performed with mean  $\pm$  SEM of fold stimulation by IGF-1 of at least 3 independently performed experiments. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0123385>), licensed under a CC-BY license.

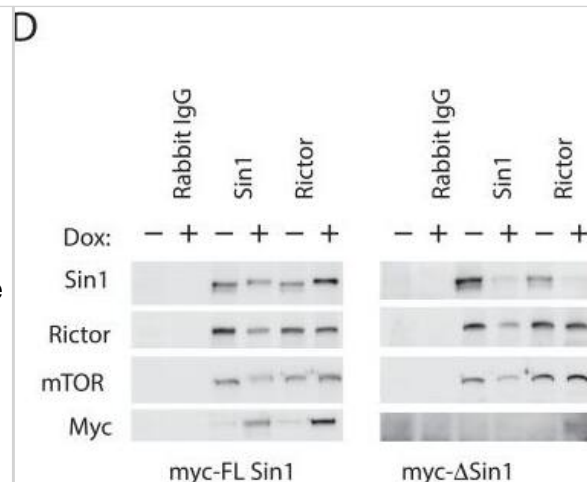


**Western Blot: Rictor Antibody [NB100-612] - Truncated Sin1 displaces endogenous Sin1 from mTORC2 in DLD1 colon cancer cells.**

**A.** Schematic indicating the domain structure of Sin1 & the constructs used to displace endogenous Sin1 from mTORC2. **B.** Expression of myc tagged Sin1 constructs can be detected only after induction with Doxycycline (Dox). Cells were treated with 100nM of doxycycline (+) for 72 hours & expressed proteins were detected by immunoblot of whole cell lysates with anti-myc (9E10) antibodies. **C. & D.** Sin1 constructs incorporate into mTORC2 & displace endogenous Sin1. Constructs were induced for 72 hours prior to immune precipitation. **(C)** mTORC2 subunits, mTOR & Rictor, only appear in myc immunoprecipitates after induction with doxycycline (Left panels); myc- $\Delta$ Sin1 cannot be directly detected in precipitates due to secondary antibody cross reaction with precipitating IgG. Right panels indicate unchanging expression levels of Rictor & mTOR in immune precipitation input lysates, which is further quantified from 3 independent experiments **E.** Endogenous Sin1 & Rictor immunoprecipitates demonstrate displacement of endogenous Sin1 from mTORC2. Following induction, band shifted myc-tagged FL Sin1 can be detected in Sin1 & Rictor precipitates (Left panels). Truncated  $\Delta$ Sin1 can be detected in Rictor, but not Sin1, immunoprecipitates as the Sin1 antibody epitope is deleted from  $\Delta$ Sin1. **F.** Quantification of Sin1 levels detected in Rictor immunoprecipitates indicates the level of endogenous mTORC2 disruption following Sin1 construct induction (data are mean  $\pm$  S.D; n = 3). Myc- $\Delta$ Sin1 displaces >80% of endogenous Sin1 while levels of myc-FL Sin1 associated with Rictor are comparable with endogenous Sin1 levels. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.20086>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Rictor Antibody [NB100-612] - Truncated Sin1 displaces endogenous Sin1 from mTORC2 in DLD1 colon cancer cells. A. Schematic indicating the domain structure of Sin1 & the constructs used to displace endogenous Sin1 from mTORC2. B. Expression of myc tagged Sin1 constructs can be detected only after induction with Doxycycline (Dox). Cells were treated with 100nM of doxycycline (+) for 72 hours & expressed proteins were detected by immunoblot of whole cell lysates with anti-myc (9E10) antibodies. C. & D. Sin1 constructs incorporate into mTORC2 & displace endogenous Sin1. Constructs were induced for 72 hours prior to immune precipitation. (C) mTORC2 subunits, mTOR & Rictor, only appear in myc immunoprecipitates after induction with doxycycline (Left panels); myc- $\Delta$ Sin1 cannot be directly detected in precipitates due to secondary antibody cross reaction with precipitating IgG. Right panels indicate unchanging expression levels of Rictor & mTOR in immune precipitation input lysates, which is further quantified from 3 independent experiments. E. Endogenous Sin1 & Rictor immunoprecipitates demonstrate displacement of endogenous Sin1 from mTORC2. Following induction, band shifted myc-tagged FL Sin1 can be detected in Sin1 & Rictor precipitates (Left panels). Truncated  $\Delta$ Sin1 can be detected in Rictor, but not Sin1, immunoprecipitates as the Sin1 antibody epitope is deleted from  $\Delta$ Sin1. F. Quantification of Sin1 levels detected in Rictor immunoprecipitates indicates the level of endogenous mTORC2 disruption following Sin1 construct induction (data are mean  $\pm$  S.D; n = 3). Myc- $\Delta$ Sin1 displaces >80% of endogenous Sin1 while levels of myc-FL Sin1 associated with Rictor are comparable with endogenous Sin1 levels. Image collected & cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.20086>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





## Publications

Gerlach BD, Ampomah PB, Yurdagul A Et al. Efferocytosis induces macrophage proliferation to help resolve tissue injury Cell metabolism 2021-11-10 [PMID: 34784501]

Ko J ALTERNATIVE POLYADENYLATION MODULATES EXPRESSION OF PRO-FIBROTIC PROTEINS AND CONTRIBUTES TO LUNG FIBROSIS Thesis 2020-01-01 (WB, Human)

Ko J, Mills T, Huang J et al. Transforming growth factor beta 1 alters the 3'UTR of mRNA to promote lung fibrosis J. Biol. Chem. [PMID: 31488543] (WB)

Cameron AJM, Veeriah S, Marshall JJT et al. Uncoupling TORC2 from AGC kinases inhibits tumour growth Oncotarget. 2017-10-17 [PMID: 29156676] (WB, Human)

Liu WL, Yang HC, Hsu CS et al. Pegylated IFN- $\alpha$  suppresses hepatitis C virus by promoting the DAPK-mTOR pathway. Proc. Natl. Acad. Sci. U.S.A. 2016-12-20 [PMID: 27930338] (WB, Human)

Tsai JS, Chao CH, Lin LY. Cadmium Activates Multiple Signaling Pathways That Coordinately Stimulate Akt Activity to Enhance c-Myc mRNA Stability. PLoS ONE. 2016-01-12 [PMID: 26751215] (WB, Human)

Kusch A, Schmidt M, Gorgen D et al. 17-Beta-Estradiol Regulates mTORC2 Sensitivity to Rapamycin in Adaptive Cardiac Remodeling PLoS ONE 2015-04-17 [PMID: 25880554] (WB, Mouse)

Smrz D, Cruse G, Beaven Ma et al. Rictor negatively Regulates High-Affinity Receptors for IgE-Induced Mast Cell Degranulation J. Immunol. 2014-11-05 [PMID: 25378594] (ICC/IF, Human)

### Details:

Rictor antibody used for ICC-IF on LAD2 human mast cell line (FIGURE 1).

Shanmugasundaram K, Block K, Nayak BK et al. PI3K regulation of the SKP-2/p27 axis through mTORC2. Oncogene 2012-06-01 [PMID: 22733130]

Gulhati P, Bowen KA, Liu J et al. mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. Cancer Res 2011-05-01 [PMID: 21430067]

Siegel N, Rosner M, Unbekandt M et al. Contribution of human amniotic fluid stem cells to renal tissue formation depends on mTOR. Hum Mol Genet 2010-09-01 [PMID: 20542987]

Gulhati P, Cai Q, Li J et al. Targeted inhibition of mammalian target of rapamycin signaling inhibits tumorigenesis of colorectal cancer. Clin Cancer Res 2009-12-01 [PMID: 19934294]

More publications at <http://www.novusbio.com/NB100-612>





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### **Products Related to NB100-612**

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NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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