## **Product Datasheet**

### Sin1/MAPKAP1 Antibody - BSA Free NB110-40424

Unit Size: 100 ul

Store at 4C. Do not freeze.

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#### NB110-40424

Sin1/MAPKAP1 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	59 kDa	
Product Description		
Host	Rabbit	
Gene ID	79109	
Gene Symbol	MAPKAP1	
Species	Human	
Reactivity Notes	Orangutan (100%).	
Immunogen	The immunogen recognized by this antibody maps to a region between residue 470 and the C-terminus (residue 522) of human stress-activated map kinase interacting protein 1 using the numbering given in entry NP_001006618.1 (GeneID 79109).	
Product Application Details		
Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation	
Recommended Dilutions	Western Blot 1:2000-1:10000, Immunohistochemistry 1:1000 - 1:5000, Immunoprecipitation 2-5 ug/mg lysate, Immunohistochemistry-Paraffin 1:1000- 1:5000	
Application Notes	Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.	

#### Images

Western Blot: Sin1/MAPKAP1 Antibody [NB110-40424] - Truncated Sin1/MAPKAP1 displaces endogenous Sin1/MAPKAP1 from mTORC2 in DLD1 colon cancer cells. Sin1/MAPKAP1 constructs incorporate into mTORC2 and displace endogenous Sin1/MAPKAP1. Constructs were induced for 72 hours prior to immune precipitation. mTORC2 subunits, mTOR and Rictor, only appear in myc immunoprecipitates after induction with doxycycline (Left panels); myc-deltaSin1/MAPKAP1 cannot be directly detected in precipitates due to secondary antibody cross reaction with precipitating IgG. Right panels indicate unchanging expression levels of Rictor and mTOR in immune precipitation input lysates, which is further quantified from 3 independent experiments. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/fulltext/20086), licensed under a CC-BY license.





Immunohistochemistry-Paraffin: Sin1/MAPKAP1 Antibody [NB110- 40424] - Section of human prostate carcinoma. Antibody: Affinity purified rabbit anti- Sin1 used at a dilution of 1:1,000 (1ug/ml). Detection: DAB	
Western Blot: Sin1/MAPKAP1 Antibody [NB110-40424] - Detection of Human Sin1 on HeLa whole cell lysate.	A WB B IP/WB kDa 250- 130- 70- 70- 51- 51- 51- 51- 51- 51- 51- 51
Truncated Sin1 displaces endogenous Sin1 from mTORC2 in DLD1 colon cancer cellsA. 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 due to secondary antibody cross reaction with precipitating lgG. 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 as the 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 Sin1 while levels of myc-ASin1 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.	C D



Truncated Sin1 displaces endogenous Sin1 from mTORC2 in DLD1 x colon cancer cellsA. 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-∆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 ∆Sin1. F. Quantification of Sin1 levels detected in Rictor immunoprecipitates indicates the level of endogenous mTORC2 disruption following Sin1 construct induction (data are mean +/- 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),

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#### **Publications**

Wang Q, Zhu J et al. Tumor suppressor Pdcd4 attenuates Sin1 translation to inhibit invasion in colon carcinoma. Oncogene 2017-09-11 [PMID: 28692058] (WB, Human)

Huang Y, Feng G, Cai J et al. Sin1 promotes proliferation and invasion of prostate Cancer cells by modulating mTORC2-AKT and AR signaling cascades Life Sci. 2020-02-20 [PMID: 32088212] (IF/IHC)

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

Das F, Ghosh-Choudhury N, Dey N et al. Unrestrained mammalian target of rapamycin complexes 1 and 2 increase expression of phosphatase and tensin homolog deleted on chromosome 10 to regulate phosphorylation of Akt kinase. J Biol Chem 2012-02-01 [PMID: 22184110]

Misra UK, Pizzo SV. Upregulation of mTORC2 activation by the selective agonist of EPAC, 8-CPT-2Me-cAMP, in prostate cancer cells: assembly of a multiprotein signaling complex. J Cell Biochem 2012-05-01 [PMID: 22173835]

Lu M, Wang J, Ives HE et al. mSIN1 protein mediates SGK1 protein interaction with mTORC2 protein complex and is required for selective activation of the epithelial sodium channel. J Biol Chem 2011-09-01 [PMID: 21757730]

Huang J, Wu S, Wu CL et al. Signaling events downstream of mammalian target of rapamycin complex 2 are attenuated in cells and tumors deficient for the tuberous sclerosis complex tumor suppressors. Cancer Res 2009-08-01 [PMID: 19602587]





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#### Products Related to NB110-40424

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

#### 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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