

# Product Datasheet

## DEPTOR/DEPDC6 Antibody - BSA Free NBP1-49674

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP1-49674**

DEPTOR/DEPDC6 Antibody - BSA Free

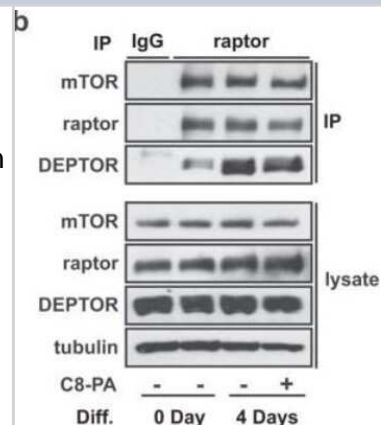
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	64798
Gene Symbol	DEPTOR
Species	Human, Mouse, Rat, Bovine
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 31176743).
Immunogen	A genomic peptide made to an internal region of the human DEPTOR protein (within residues 10-200). [Swiss-Prot: Q8TB45]
Notes	Manufactured by Genomic Antibody Technology™. GAT <a href="#">FAQs</a>

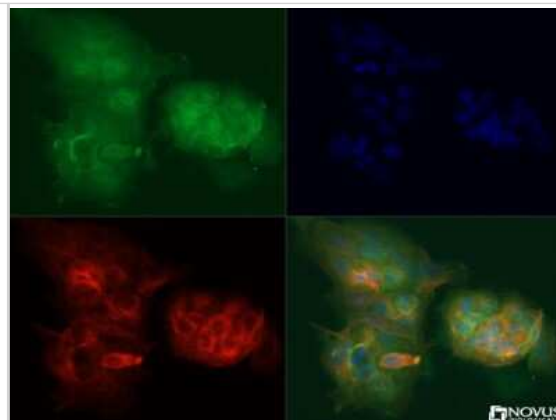
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin 1:100, Knockout Validated
Application Notes	In Western Blot, a band is seen at ~46 kDa. In ICC/IF, cytoplasmic staining was observed in serum starved HeLa cells. In IHC-P, staining was observed in the cytoplasm of mouse spleen. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

**Images**

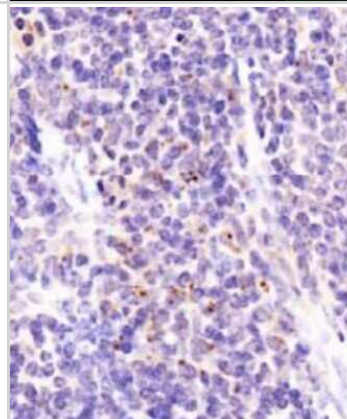
Western Blot: DEPTOR/DEPDC6 Antibody - BSA Free [NBP1-49674] - PA regulates adipogenic differentiation by displacing DEPTOR from mTORC1. 3T3-L1 preadipocytes were transfected with a PPRE luciferase reporter plasmid vector, induced to differentiate for 3 h, and then luciferase activity was measured. 3T3-L1 cells were differentiated in the absence or presence of 300 uM C8-PA. Cell lysates and immunoprecipitates against raptor were analyzed by western blotting. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep36968>), licensed under a CC-BY license.



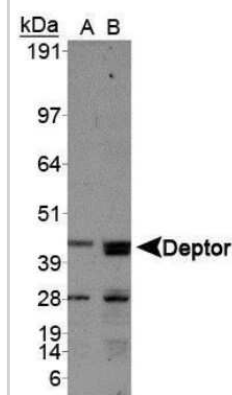
Immunocytochemistry/Immunofluorescence: DEPTOR/DEPDC6 Antibody - BSA Free [NBP1-49674] - Antibody was tested in MCF7 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



Immunohistochemistry: DEPTOR/DEPDC6 Antibody - BSA Free [NBP1-49674] - Staining of DEPTOR in mouse spleen using DAB with a hematoxylin counterstain.



Western Blot: DEPTOR/DEPDC6 Antibody - BSA Free [NBP1-49674] - Analysis of DEPDC6 in (A) human liver and (B) mouse liver.



Knockout Validated: DEPTOR/DEPDC6 Antibody - BSA Free [NBP1-49674] - Whole-body DEPTOR null mice are viable, fertile, normal in size, and do not display any gross physical abnormalities. Western blot showing the expression of DEPTOR/DEPDC6 in tissues of Deptor wild-type (wt/wt), heterozygote (Delta/wt) or knockout (Delta/Delta) mice. Akt was used as a loading control. Image collected and cropped by CiteAb from the following publication ([//pubmed.ncbi.nlm.nih.gov/28462079/](https://pubmed.ncbi.nlm.nih.gov/28462079/)) licensed under a CC-BY license.



## Publications

Marina Serrano-Maciá, Jorge Simón, Maria J. González-Rellán, Mikel Azkargorta, Naroa Goikoetxea-Usandizaga, Fernando Lopitz-Otsoa, Diego Saenz De Urturi, Rubén Rodríguez-Agudo, Sofia Lachiondo-Ortega, Maria Mercado-Gomez, Virginia Gutiérrez de Juan, Maider Bizkarguenaga, David Fernández-Ramos, Xabier Buque, Guido A. Baselli, Luca V.C. Valenti, Paula Iruzubieta, Javier Crespo, Erica Villa, Jesus M. Banales, Matias A. Avila, Jose J.G. Marin, Patricia Aspichueta, James Sutherland, Rosa Barrio, Ugo Mayor, Félix Elortza, Dimitris P. Xirodimas, Rubén Nogueiras, Teresa C. Delgado, María Luz Martínez-Chantar Neddylation inhibition ameliorates steatosis in NAFLD by boosting hepatic fatty acid oxidation via the DEPTOR-mTOR axis *Molecular Metabolism* 2021-06-18 [PMID: 34153521]

Read, TA;Cisterna, BA;Skruher, K;Ahmadieh, S;Lindamood, HL;Vitriol, JA;Shi, Y;Lefebvre, AEYT;Black, JB;Butler, MT;Bear, JA;Cherezova, A;Ilatovskaya, DV;Weintraub, NL;Vitriol, EA; The actin binding protein profilin 1 is critical for mitochondria function *bioRxiv : the preprint server for biology* 2023-08-16 [PMID: 37609280] (Western Blot)

N Saito, J Araya, S Ito, K Tsubouchi, S Minagawa, H Hara, A Ito, T Nakano, Y Hosaka, A Ichikawa, T Kadota, M Yoshida, Y Fujita, H Utsumi, Y Kurita, K Kobayashi, M Hashimoto, H Wakui, T Numata, Y Kaneko, H Asano, M Odaka, T Ohtsuka, T Morikawa, K Nakayama, K Kuwano Involvement of Lamin B1 Reduction in Accelerated Cellular Senescence during Chronic Obstructive Pulmonary Disease Pathogenesis *J. Immunol.*, 2019-01-28;0(0):. 2019-01-28 [PMID: 30692212] (B/N)

Colas C, Mouchiroud M, Al Dow M et al. DEPTOR loss impairs brown adipocyte development in vitro but has limited impacts in mice *Molecular metabolism* 2022-12-16 [PMID: 36535626] (WB, Mouse)

### Details:

Dilution used in WB 1:1000

Tan M, Chang Y, Liu X et al. The Sag-Shoc2 axis regulates conversion of mPanINs to cystic lesions in Kras pancreatic tumor model *Cell reports* 2022-12-20 [PMID: 36543126] (IHC-P, Mouse)

### Details:

Dilution used in IHC-P 1:400

George Sa, Kiss A, Obaid Sn et Al. p38delta genetic ablation protects female mice from anthracycline cardiotoxicity *Am J Physiol Heart Circ Physiol* 2020-08-22 [PMID: 32822209]

Gnanapradeepan K, Basu S, Barnoud T et al. Increased mTOR activity and metabolic efficiency in mouse and human cells containing the African-centric tumor-predisposing p53 variant Pro47Ser *Elife* 2020-11-10 [PMID: 33170774]

Ruiz-Serrano A, Boyle CN, MonnE Rodrlguez JM et al. The Deubiquitinase OTUB1 Is a Key Regulator of Energy Metabolism *International journal of molecular sciences* 2022-01-28 [PMID: 35163456] (WB, Mouse)

Ruiz-Serrano A, MonnE Rodrlguez JM, GUnter J Et al. OTUB1 regulates lung development, adult lung tissue homeostasis, and respiratory control *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2021-12-01 [PMID: 34793600] (WB, Human)

De Carvalho F, Munoz V, Brandao C Et Al. Taurine upregulates insulin signaling and mitochondrial metabolism in vitro, but not in adipocytes of obese women *Nutrition* 2021-09-03 [PMID: 34479044] (WB)

Kim D, Park G, Huuhtanen J et al. Somatic mTOR mutation in clonally expanded T lymphocytes associated with chronic graft versus host disease *Nat Commun* 2020-05-07 [PMID: 32382059] (WB, Human)

Baek MO, Ahn CB, Cho HJ et al. Simulated microgravity inhibits C2C12 myogenesis via phospholipase D2-induced Akt/FOXO1 regulation *Sci Rep* 2019-10-17 [PMID: 31624287] (WB, Mouse)

More publications at <http://www.novusbio.com/NBP1-49674>



## Procedures

### Western Blot protocol specific for Deptor Antibody (NBP1-49674)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute the rabbit anti-Deptor primary antibody (NBP1-49674) in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

\*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### Immunohistochemistry-Paraffin protocol for DEPTOR/DEPDC6 Antibody (NBP1-49674)

#### Immunohistochemistry-Paraffin Embedded Sections

##### Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

##### Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

**Immunocytochemistry/Immunofluorescence Protocol for DEPTOR Antibody (NBP1-49674)**

## Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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### **Products Related to NBP1-49674**

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NB820-59662	Mouse Liver Whole Tissue Lysate (Adult Whole Normal)
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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