

Product Datasheet

DEPTOR/DEPDC6 Antibody

NBP1-49674SS

Unit Size: 0.025 ml

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

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NBP1-49674SS

DEPTOR/DEPDC6 Antibody

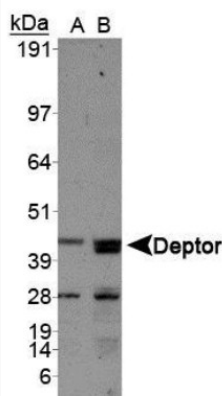
Product Information	
Unit Size	0.025 ml
Concentration	1.25 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	64798
Gene Symbol	DEPTOR
Species	Human, Mouse
Species Reactivity	Human and mouse. Predicted to react with bovine based on 100% sequence homology.
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 FAQs

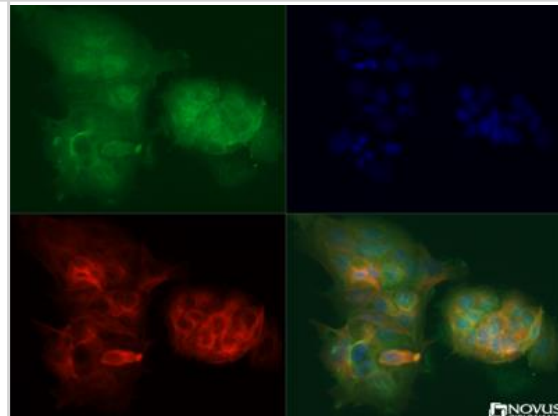
Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:100, Immunohistochemistry 1:100, Immunohistochemistry-Paraffin 1:100, Western Blot 1:1000
Application Notes	This DEPTOR antibody is useful for Immunocytochemistry/Immunofluorescence, Western Blot, and Immunohistochemistry-paraffin embedded sections. 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

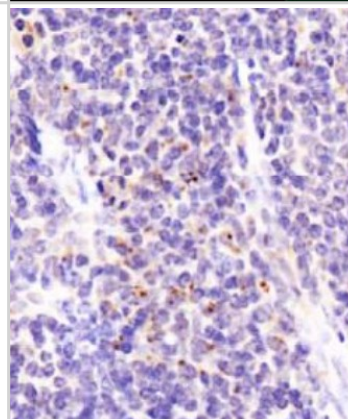
Western Blot: DEPTOR Antibody [NBP1-49674] - Analysis of DEPDC6 in A. human liver and B. mouse liver.



Immunocytochemistry/Immunofluorescence: DEPTOR Antibody [NBP1-49674] - DEPTOR 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 Antibody [NBP1-49674] - Staining of DEPTOR in mouse spleen using DAB with a hematoxylin counterstain.



Publications

Caron A, Baraboi ED, Laplante M et al. DEP domain-containing mTOR-interacting protein in the rat brain: distribution of expression and potential implication *J. Comp. Neurol.* 2014 Aug 27 [PMID: 25159114]

Brundage ME, Tandon P, Eaves DW et al. MAF mediates crosstalk between Ras-MAPK and mTOR signaling in NF1. *Oncogene* 2014 Feb 10 [PMID: 24509877] (WB, ICC/IF, Human)

Fernandez-Saiz V, Targosz BS, Lemeer S et al. SCF(Fbxo9) and CK2 direct the cellular response to growth factor withdrawal via Tel2/Tti1 degradation and promote survival in multiple myeloma *Nat Cell Biol* 2012 Dec 23 [PMID: 23263282] (WB, Human)

Jegg AM, Ward TM, Iorns E et al. PI3K independent activation of mTORC1 as a target in lapatinib-resistant ERBB2+ breast cancer cells *Breast Cancer Res Treat* 2012 Oct 23 [PMID: 23089982] (WB, Human)

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 Embedded Sections Protocol (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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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.

