

Product Datasheet

WDFY3 Antibody - BSA Free

NBP1-03332

Unit Size: 0.1 mg

Store at -20 degrees C. Avoid freeze/thaw cycles.

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NBP1-03332

WDFY3 Antibody - BSA Free

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

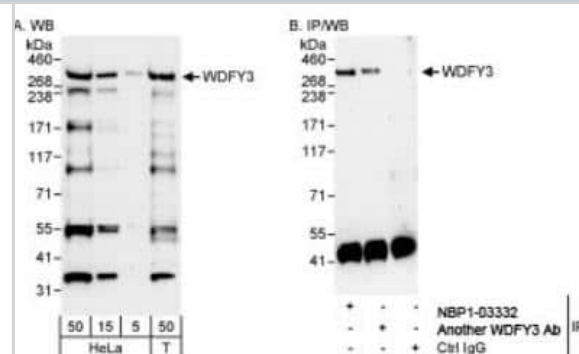
Product Description	
Host	Rabbit
Gene ID	23001
Gene Symbol	WDFY3
Species	Human, Mouse
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 27330028).
Immunogen	Synthetic peptide made to an internal portion of the human WDFY3 protein (between amino acids 500-550) [Q8IZQ1]

Product Application Details	
Applications	Western Blot, Electron Microscopy, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:2000-1:10000, Immunohistochemistry 1:200 - 1:1000, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunoprecipitation 2-5 mcg/mg lysate, Immunohistochemistry-Paraffin 1:200 - 1:1000, Electron Microscopy
Application Notes	Use in Electron Microscopy reported in scientific literature (PMID 24358206) Use in Immunocytochemistry/immunofluorescence reported in scientific literature (PMID 24664425). Immunohistochemistry 1:1,000 to 1:5,000. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections

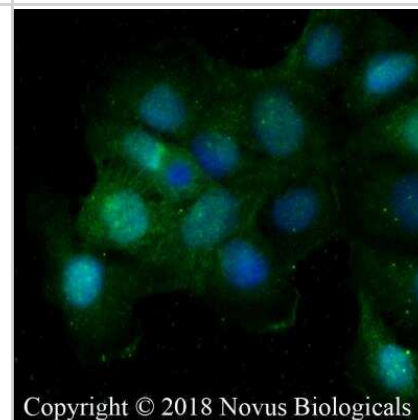


Images

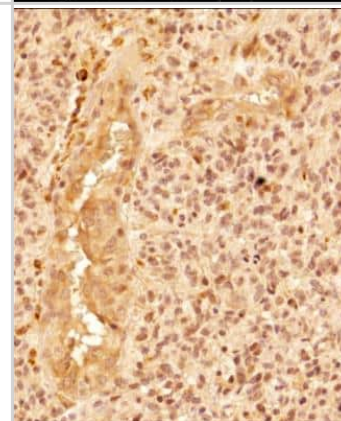
Western Blot: WDFY3 Antibody [NBP1-03332] - Whole cell lysate from HeLa (5, 15 and 50 mcg for WB; 1 mg for IP, 20% of IP loaded) and 293T (T; 50 mcg) cells. NBP1-03332 used for WB at 1 mcg/ml (A and B) and used for IP at 3 mcg/mg lysate.



Immunocytochemistry/Immunofluorescence: WDFY3 Antibody [NBP1-03332] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-WDFY3 at 5 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry-Paraffin: WDFY3 Antibody [NBP1-03332] - IHC analysis of a formalin fixed paraffin embedded tissue section of the human glioblastoma using 1:200 dilution of WDFY3 antibody (NBP1-03332). The signal was developed using HRP-DAB method which followed counterstaining of the cells with hematoxylin



Publications

Soreng K, Pankiv S, Bergsmark C et al. ALFY localizes to early endosomes and cellular protrusions to facilitate directional cell migration *Journal of cell science* 2022-02-15 [PMID: 35099014] (WB)

Wu DJ, Adamopoulos IE. Loss of WDFY3 ameliorates severity of serum transfer-induced arthritis independently of autophagy. *Cell Immunol* 2017-04-22 [PMID: 28449847]

Suzuki E, Maverakis E, Sarin R et al. T Cell-Independent Mechanisms Associated with Neutrophil Extracellular Trap Formation and Selective Autophagy in IL-17A-Mediated Epidermal Hyperplasia. *J. Immunol.* 2016-10-24 [PMID: 27798153] (ICC/IF, WB)

Wu DJ, Gu R, Sarin R et al. Autophagy-linked FYVE containing protein WDFY3 interacts with TRAF6 and modulates RANKL-induced osteoclastogenesis. *J. Autoimmun.* 2016-06-18 [PMID: 27330028] (IP, ICC/IF, WB, Mouse)

Karanasios E, Ktistakis NT. Studying Autophagy: List of Useful Antibodies Produced via a Community Effort. *Autophagy at the Cell, Tissue and Organismal Level.* 2016-04-24

Orosco LA, Ross AP, Cates SL et al. Loss of Wdfy3 in mice alters cerebral cortical neurogenesis reflecting aspects of the autism pathology. *Nat Commun.* 2014-09-08 [PMID: 25198012]

Park S, Jang I, Zuber C et al. ERADication of EDEM1 occurs by selective autophagy and requires deglycosylation by cytoplasmic peptide N-glycanase. *Histochem. Cell Biol.* 2014-03-25 [PMID: 24664425] (EM, ICC/IF, Human)

Sommi P, Necchi V, Vitali A et al. PaCS Is a Novel Cytoplasmic Structure Containing Functional Proteasome and Inducible by Cytokines/Trophic Factors. *PLoS One* 2013-12-17 [PMID: 24358206] (EM, Human)



Procedures

Western Blot protocol for WDFY3 Antibody (NBP1-03332)

WDFY3 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute anti-ARG1 primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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.

Immunocytochemistry/Immunofluorescence protocol for WDFY3 Antibody (NBP1-03332)

WDFY3 Antibody:

Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

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



Immunohistochemistry-Paraffin protocol for WDFY3 Antibody (NBP1-03332)

WDFY3 Antibody:

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 4 C.
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.





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