

Product Datasheet

Derlin 1 Antibody - BSA Free NB100-448

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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NB100-448

Derlin 1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)
Target Molecular Weight	29 kDa

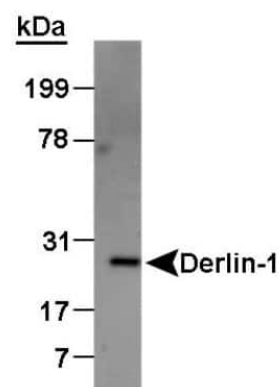
Product Description	
Description	Novus Biologicals Rabbit Derlin 1 Antibody - BSA Free (NB100-448) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. Anti-Derlin 1 Antibody: Cited in 2 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	79139
Gene Symbol	DERL1
Species	Human, Mouse, Canine, Chinese Hamster
Reactivity Notes	Predicted to react with Xenopus, bovine, chicken, rat, zebrafish, and mouse based on 100% sequence homology. Mouse reactivity reported in scientific literature (PMID: 25377857)
Immunogen	A synthetic peptide made to the C-terminal region of human Derlin 1 (between residues 200-251). [UniProt# Q9BUN8]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1:1000-1:5000, Immunohistochemistry 5-10 ug/ml, Immunocytochemistry/ Immunofluorescence 1:2000, Immunohistochemistry-Paraffin 5-10 ug/ml
Application Notes	In Western blot, a band is seen at ~29 kDa. In ICC/IF, ER staining was observed in HeLa cells. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

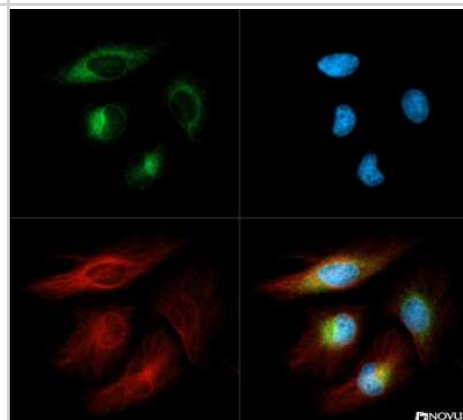


Images

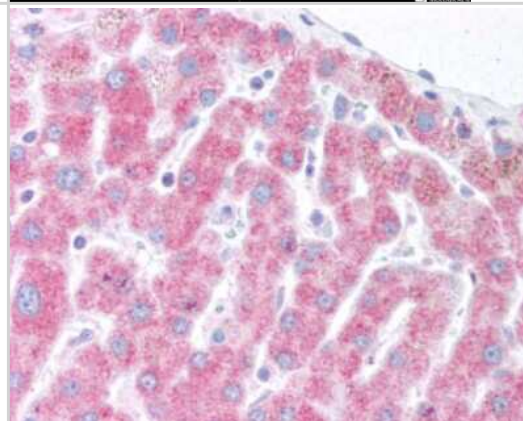
Western Blot: Derlin 1 Antibody [NB100-448] - Detection of Derlin protein in canine KRM microsomes.



Immunocytochemistry/Immunofluorescence: Derlin 1 Antibody [NB100-448] - Derlin-1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry: Derlin 1 Antibody [NB100-448] - Staining of human liver.



Publications

Sugawara T, Kano F, Murata M. Rab2A is a pivotal switch protein that promotes either secretion or ER-associated degradation of (pro)insulin in insulin-secreting cells Sci Rep et al. 2014-11-07 [PMID: 25377857] (ICC/IF, WB, Mouse)

Details:

Derlin-1 antibody used for ICC-IF on MIn6 cells expressing GFP-p58 or GFP-p58 and Myc-Rab2A (Figure 2d and h). Antibody also used for WB on 1% Tx soluble and LUb-ERGIC enriched fractions of MIn6 cells (murine pancreatic beta cell line) transfected with scramble or Rab2A siRnA (Figure 4a).

Huttunen HJ, Guenette SY, Peach C et al. HtrA2 regulates beta-amyloid precursor protein (APP) metabolism through endoplasmic reticulum-associated degradation. J Biol Chem 2007-09-01 [PMID: 17684015] (WB, Chinese Hamster)

Procedures

Serum protocol for Derlin 1 Antibody (NB100-448)

Derlin 1 Antibody:

Western Blot

1. Run samples on a 10-15% SDS-PAGE.
2. Transfer proteins to a methanol soaked PVDF membrane for 2.5 hr at 250 milliamper at 4C on a stir plate.
3. Immerse PVDF membrane in blocking buffer and block for 1 hour at room temperature.
4. Wash membrane 3 times with TBS-T.
5. Incubate with primary Derlin-1 antibody (NB 100-448), diluted in blocking buffer 1:500, overnight at 4C
6. Wash membrane 3 x 10 min with TBS-T.
7. Incubate with secondary antibody, diluted in blocking buffer, at room temp for 1 hour.
8. Wash 3 x 10 min with TBS-T.
9. Detect with Amersham ECL kit (RPN 2106).

Buffers

10x Blotting buffer: 1 L

30.3 g Trizma base (= 0.25 M)

144 g Glycine (= 1.92 M)

pH should be 8.3; do not adjust

To make 2 L of 1x Blotting buffer:

400 ml Methanol

200 ml 10x Blotting buffer

1400 ml water

TBS-T:

50 mM TrisHCl

150 mM NaCl pH 7.4

0.05% Tween-20

Blocking buffer:

50 mM TrisHCl

150 mM NaCl pH 7.4

0.05% Tween-20

3% milk powder



IHC-FFPE sections

I. Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes.

To Prepare 200 ml of Quenching Solution:

Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.

Use within 4 hours of preparation

B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.

B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.

C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.

D. Slowly add distilled water to further cool for 5 minutes.

E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).

B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.

D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.

E. Wash slides with Wash Solution: 3 changes for 5 minutes each.

F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.

G. Wash slides with Wash Solution: 3 changes for 5 minutes each.

H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.

I. Wash slides with Wash Solution: 3 changes for 5 minutes each.

J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker

counterstaining is desired.

K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.

L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.

M. Rinse slides in distilled water.

N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.

O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.

P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.

- Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.

- All steps in which Xylene is used should be performed in a fume hood.

- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).



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Products Related to NB100-448

NBL1-09837	Derlin 1 Overexpression Lysate
NB100-448PEP	Derlin 1 Antibody Blocking Peptide
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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