

Product Datasheet

HRD1 Antibody - BSA Free NB100-2526

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB100-2526

HRD1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

Description	Novus Biologicals Knockout (KO) Validated Rabbit HRD1 Antibody - BSA Free (NB100-2526) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and IP. Anti-HRD1 Antibody: Cited in 15 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	84447
Gene Symbol	SYVN1
Species	Human, Mouse, Hamster
Reactivity Notes	Hamster reactivity reported in scientific literature (PMID: 25078082).
Immunogen	A synthetic peptide made to an internal portion of human HRD1 (within residues 300-400). [UniProt Q96PK3]

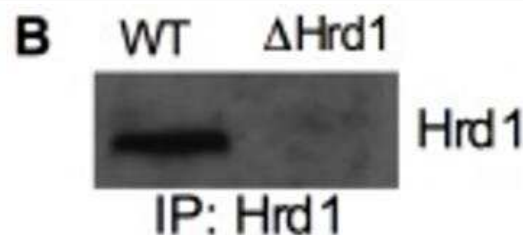
Product Application Details

Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1:1000 - 1:2000, Immunohistochemistry 1:200 - 1:500, Immunocytochemistry/ Immunofluorescence 1:500, Immunoprecipitation reported in scientific literature (PMID 35350536), Immunohistochemistry-Paraffin 1:200 - 1:500. Use reported in scientific literature (PMID 24453213), Knockout Validated reported in scientific literature (PMID 26551274)
Application Notes	Heat samples at 50C for 30 minutes to detect HRD1 by Western blot.

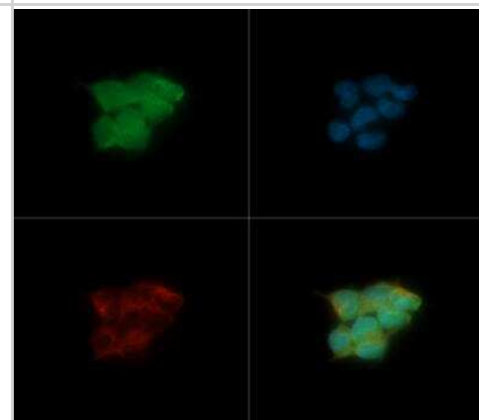


Images

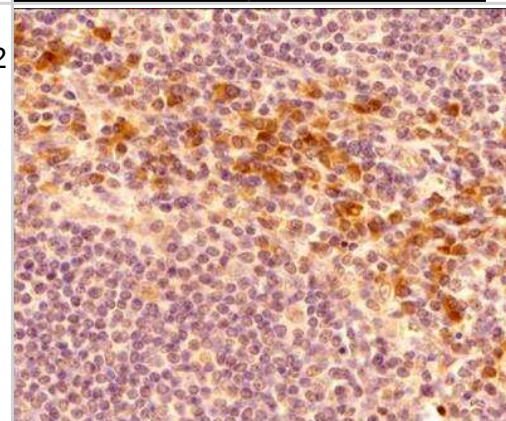
Western Blot: HRD1 Antibody [NB100-2526] - Hrd1 is required for CDT intoxication. Co-immunoprecipitation of Derl2 and Hrd1. Derl2 was immunoprecipitated and samples were analyzed for Hrd1 by western blot. Image collected and cropped by CiteAb from the following publication ([//doi.org/10.1371/journal.ppat.1004295](https://doi.org/10.1371/journal.ppat.1004295)) licensed under a CC-BY license.



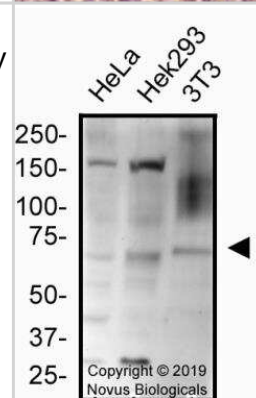
Immunocytochemistry/Immunofluorescence: HRD1 Antibody [NB100-2526] - HRD1 antibody was tested in Hek293 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry-Paraffin: HRD1 Antibody [NB100-2526] - FFPE tissue section of human tonsil using rabbit polyclonal HRD1 antibody at 2 ug/mL concentration (1:500 dilution). The primary antibody binding to SYVN1/HRD1 antigen in cells was detected using HRP conjugated anti-Rabbit secondary antibody with DAB reagent, and the sections were further counterstained with hematoxylin for labeling cellular nuclei. This HRD1 antibody generated an expected cytoplasmic staining of HRD1 with stronger signal around periphery of the nucleus signifying the staining of the endoplasmic reticulum.



Western Blot: HRD1 Antibody [NB100-2526] - Total protein from human HeLa, Hek293 and mouse 3T3 cell lines was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-HRD1 in block buffer and detected with an anti-rabbit HRP secondary antibody using West Pico PLUS chemiluminescence detection reagent.



Publications

Lee ES, Aryal YP, Kim TY et al. The effects of 4-Phenylbutyric acid on ER stress during mouse tooth development *Frontiers in Physiology* 2023-01-04 [PMID: 36685173] (Western Blot, Human)

Sarah Poliquin, Gerald Nwosu, Karishma Randhave, Wangzhen Shen, Carson Flamm, Jing-Qiong Kang, Henry Hing Cheong Lee Modulating Endoplasmic Reticulum Chaperones and Mutant Protein Degradation in GABRG2(Q390X) Associated with Genetic Epilepsy with Febrile Seizures Plus and Dravet Syndrome *International Journal of Molecular Sciences* 2024-04-23 [PMID: 38731820]

Lee E, Aryal Y, Kim T et al. Facilitation of Reparative Dentin Using a Drug Repositioning Approach With 4-Phenylbutyric Acid *Front Physiol* 2022-05-23 [PMID: 35600310]

Binder P, Nguyen B, Collins L et al. Pak2 Regulation of Nrf2 Serves as a Novel Signaling Nexus Linking ER Stress Response and Oxidative Stress in the Heart *Frontiers in cardiovascular medicine* 2022-03-08 [PMID: 35350536] (IP)

Seidel E, Dassa L, Kahlon S et al. A slowly cleaved viral signal peptide acts as a protein-integral immune evasion domain *Nature communications* 2021-04-06 [PMID: 33824318] (WB, Human)

Jiang LL, Zhu B, Zhao Y et al. Membralin deficiency dysregulates astrocytic glutamate homeostasis leading to ALS-like impairment *J. Clin. Invest.* 2019-05-21 [PMID: 31112137] (IHC-P, Human, Mouse)

Xie H, Tang CA, Song JH, Mancuso A. IRE1a RNase-dependent lipid homeostasis promotes survival in Myc-transformed cancers. *J. Clin. Invest.* 2018-02-26 [PMID: 29381485] (WB, Human)

Hwang J, Walczak CP, Shaler TA et al. Characterization of protein complexes of the endoplasmic reticulum associated degradation E3 ubiquitin ligase Hrd1. *J. Biol. Chem.* 2017-04-14 [PMID: 28411238] (WB, Human)

Sun S, Shi G, Sha H et al. IRE1alpha is an endogenous substrate of endoplasmic-reticulum-associated degradation. *Nat Cell Biol* 2015-12-01 [PMID: 26551274] (WB)

Koenig Pa, Nicholls Pk, Schmidt Fi et al. The E2 Ubiquitin Conjugating Enzyme UBE2J1 is Required for Spermiogenesis in Mice. *J. Biol. Chem.* 2014-10-15 [PMID: 25320092] (WB, Mouse)

Eshraghi A, Dixon SD, Tamilselvam B et al. Cytolethal Distending Toxins Require Components of the ER-Associated Degradation Pathway for Host Cell Entry *PLoS Pathog.* 2014-07-01 [PMID: 25078082]

Sun S, Shi G, Han X et al. Sel1L is indispensable for mammalian endoplasmic reticulum-associated degradation, endoplasmic reticulum homeostasis, and survival. *Proc. Natl. Acad. Sci. U.S.A.* 2014-02-05 [PMID: 24453213] (IHC-P, WB, Mouse)

More publications at <http://www.novusbio.com/NB100-2526>



Procedures

Western Blot Protocol for HRD1 Antibody (NB100-2526)

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 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 manufacturer's instructions.

Immunocytochemistry/Immunofluorescence Protocol for HRD1 Antibody (NB100-2526)

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.



Immunohistochemistry-Paraffin Protocol for HRD1 Antibody (NB100-2526)**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 (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) 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 HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.





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Products Related to NB100-2526

NBL1-16659	HRD1 Overexpression 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

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