Product Datasheet

Ferroportin/SLC40A1 Antibody
NBP1-21502SS

Unit Size: 0.025 ml

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

Publications: 11

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Ferroportin/SLC40A1 Antibody

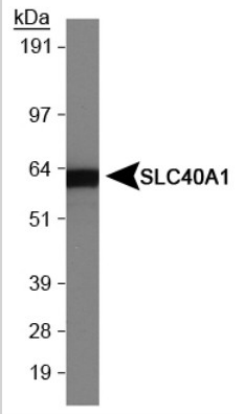
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl
Target Molecular Weight	62 kDa

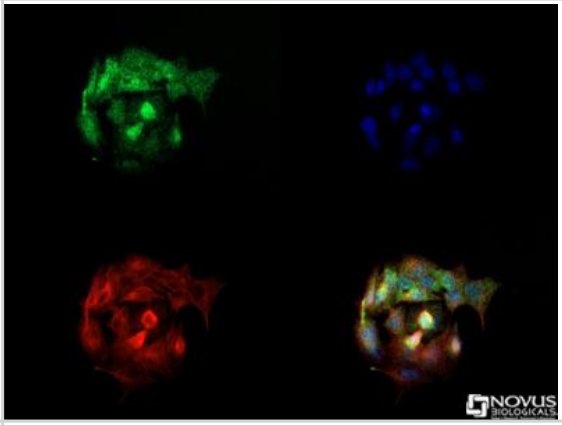
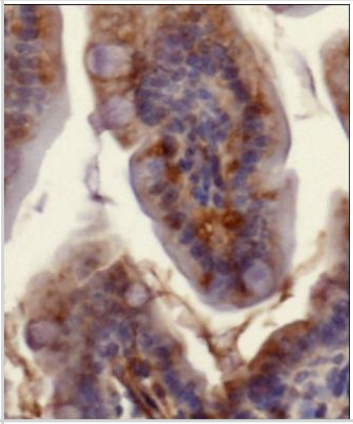
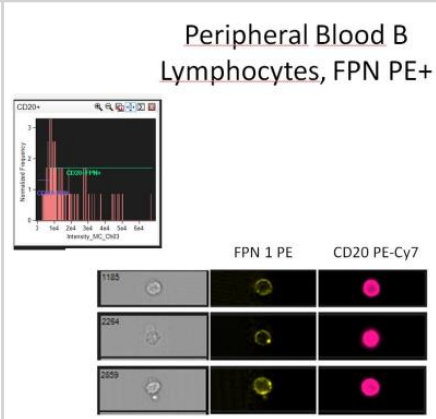
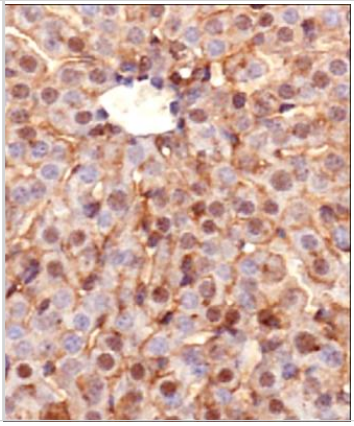
Product Description	
Host	Rabbit
Gene ID	30061
Gene Symbol	SLC40A1
Species	Human, Mouse
Species Reactivity	Human and mouse.
Immunogen	Synthetic peptide made to an internal portion of human Ferroportin 1 (within residues 250-300). [Swiss-Prot# Q9NP59]
Notes	Antibody tested in Flow Cytometry by verified customer through our Innovator's Reward Program.

Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:10, Immunocytochemistry/Immunofluorescence 1:500, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200, Western Blot 1.0 ug/ml
Application Notes	This Ferroportin 1 antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot, where a band is seen at ~62 kDa.

Images

Western Blot: Ferroportin 1 Antibody [NBP1-21502] - Human intestine lysate.



Immunocytochemistry/Immunofluorescence: Ferroportin 1 Antibody [NBP1-21502] - SLC40A1 (Ferroportin-1) antibody was tested in HepG2 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Immunohistochemistry-Paraffin: Ferroportin 1 Antibody [NBP1-21502] - IHC-P detection of FPN1 / SLC40A1 protein in murine small intestinal section using Ferroportin 1 antibody at a dilution of 1:200. The antibody primarily developed a membranous staining pattern in the intestinal epithelial cells.	
Flow Cytometry: Ferroportin 1 Antibody [NBP1-21502] - FPN1 detection on peripheral blood b lymphocytes. Data provided courtesy of a verified customer via our Innovator's Reward program.	
Immunohistochemistry-Paraffin: Ferroportin 1 Antibody [NBP1-21502] - IHC-P detection of FPN1 / SLC40A1 protein in murine liver section using Ferroportin 1 antibody at a dilution of 1:200. The representative image shows intense staining in the cellular membranes, whereas, a relatively milder postivity was observed in the cytoplasm of hepatocytes.	

Publications

Gammella E, Diaz V, Recalcati S et al. Erythropoietin's inhibiting impact on hepcidin expression occurs indirectly. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2014 Dec 17 [PMID: 25519735] (WB, Mouse)

Katz O, Reifen R, Lerner A. B-Carotene can reverse dysregulation of iron protein in an in vitro model of inflammation. Immunol. Res. 2014 Nov 15 [PMID: 25398638] (WB, Human)

Theurl M, Nairz M, Schroll A et al. Hepcidin as a predictive factor and therapeutic target in erythropoiesis-stimulating agent treatment for anemia of chronic disease in rats. Haematologica. 2014 Jun 03 [PMID: 24895335] (WB, IHC-P, Rat)

Details:
Formalin fixed and paraffin-wax embedded rat (female Lewis rats) spleen and duodenum tissue sections were immunostained with 1:200 dilutionof rabbit anti-Fp1 antibdoy (NBP1-21502) in PBS/1% BSA for 1h at RT followed by donkey anti rabbit Alexa 555 for immunofluorescence detection (Supplemental Figure S4 - Splenic Fp1 and Supplemental Figure S5 - Duodenal Fp1 immunofluorescence in ACD rats treated with ESA and/or LDN-193189). Antibody also used for WB of FP1 in spleen (Figure 3A) and liver of rats (Supplemental Figure S3).

Jeong SM, Lee J, Finley LW et al. SIRT3 regulates cellular iron metabolism and cancer growth by repressing iron regulatory protein 1. Oncogene. 2014 Jun 09 [PMID: 24909164] (WB, Mouse)

Details:
WB with Ferroportin 1 antibody on whole-cell lysates of immortalized WT and SIRT3 KO MEFs showing a decreased FPN1 protein expression in SIRT3 KO compared with WT cells (Figure 3e and Supplementary Figure S3f).

Scheers NM, Almgren AB, Sandberg AS. Proposing a Caco-2/HepG2 cell model for in vitro iron absorption studies. The Journal of Nutritional Biochemistry 3/19/2014 [PMID: 24746839] (WB, Human)

Kim DK, Jeong JH, Lee JM et al. Inverse agonist of estrogen-related receptor [gamma] controls Salmonella typhimurium infection by modulating host iron homeostasis. Nature Medicine 3/23/2014 [PMID: 24658075] (WB, Mouse)

Scheers N, Sandberg AS. Iron Transport through Ferroportin Is Induced by Intracellular Ascorbate and Involves IRP2 and HIF2alpha. Nutrients 2014 Jan 3 [PMID: 24394537] (WB, Human)

Diaz V, Gammella E, Recalcati S et al. Liver iron modulates hepcidin expression during chronically elevated erythropoiesis in mice. Hepatology 2013 Jun 6 [PMID: 23744538] (WB, Mouse)

Choi JS, Koh IU, Lee HJ et al. Effects of excess dietary iron fat on glucose lipid metabolism. J Nutr Biochem 2013 May 2 [PMID: 23643521] (WB, Mouse)

Bayeva M, Khechaduri A, Puig S et al. mTOR Regulates Cellular Iron Homeostasis through Tristetraprolin Cell Metab 2012 Oct 23 [PMID: 23102618] (WB, Mouse)

Vanoaica L et al. Intestinal ferritin h is required for an accurate control of iron absorption. Cell Metab 12(3):273-82. 2010 [PMID: 20816093] (WB, Mouse)

Procedures

Western Blot Protocol for Ferroportin 1 Antibody (NBP1-21502)
Procedure Guide for NBP1-21502 - SLC40A1 Antibody
Western Blot Protocol
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations

and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-SLC40A1 primary antibody (NBP1-21502) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the 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 [TBS + 0.1% Tween] 3 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 (Pierce ECL).
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided
it does not interfere with antibody-antigen binding.

Immunohistochemistry-Paraffin Protocol for Ferroportin 1 Antibody (NBP1-21502)

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
a. Immerse in 100% ethanol with 2 changes for 5 minutes each
b. Immerse in 95% ethanol with 2 changes for 5 minutes each
c. Immerse in 90% ethanol for 5 minutes
d. Immerse in 70% ethanol for 5 minutes
e. Immerse in 50% ethanol for 5 minutes
f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
a. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
b. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
a. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
b. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
b. Wash in deionized water for 1-2 minutes to clear the extra stain.
c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
a. 50% alcohol for 1 minute
b. 70% for 1 minute
c. 90% for 1 minute
d. 95% for 1 minute
e. 100% for 1 minute
f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.

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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.