

# Product Datasheet

## Irisin/FNDC5 Antibody - BSA Free NBP2-59680

Unit Size: 0.1 mg

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

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**NBP2-59680**

Irisin/FNDC5 Antibody - BSA Free

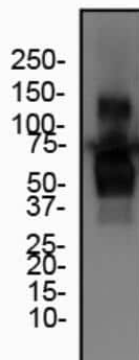
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit Irisin/FNDC5 Antibody - BSA Free (NBP2-59680) is a polyclonal antibody validated for use in IHC and WB. Anti-Irisin/FNDC5 Antibody: Cited in 3 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	252995
Gene Symbol	FNDC5
Species	Human, Mouse, Camel
Reactivity Notes	Use in Camel reported in scientific literature (PMID:33424357)
Immunogen	Partial recombinant protein made to an internal region of the human Irisin/FNDC5 protein (amino acids 32-143) [UniProt Q8NAU1]

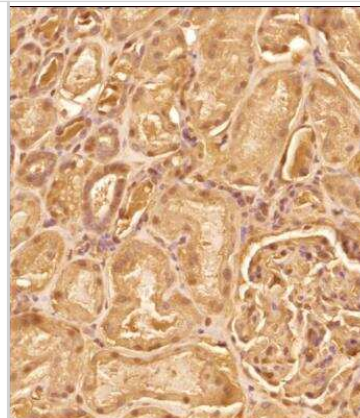
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunohistochemistry
Recommended Dilutions	Western Blot 0.5 - 1.0 ug/ml, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200
Application Notes	Irisin/FNDC5 has a theoretical molecular weight of 23 kDa, however this protein is highly glycosylated and will run higher in WB.

**Images**

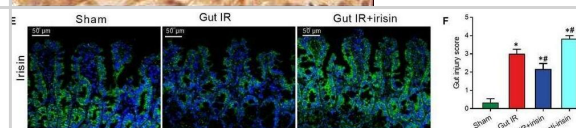
Western Blot: Irisin/FNDC5 Antibody [NBP2-59680] - Recombinant human Irisin protein was separated on a 4-20% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-Irisin/FNDC5 in block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



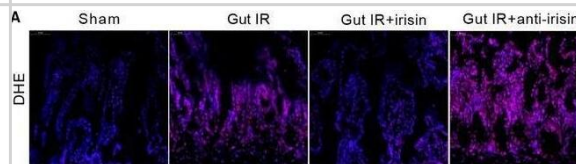
Immunohistochemistry-Paraffin: Irisin/FNDC5 Antibody [NBP2-59680] - IHC analysis of a formalin fixed and paraffin embedded human kidney tissue section (NBP1-78269) using 1:200 dilution of Irisin/FNDC5 antibody. The signal was developed with HRP-DAB detection and the sections were counterstained with hematoxylin. The antibody generated a membrane cytoplasmic staining in almost all of the cell types in the tested section.



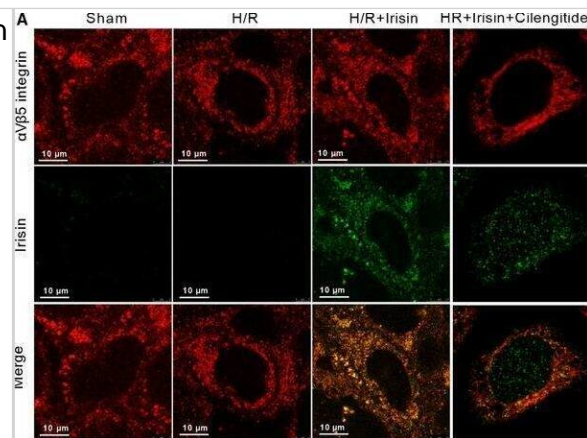
Exogenous irisin restores gut barrier function after gut IR. Irisin (250  $\mu\text{g}/\text{kg}$  in 0.5 mL saline, a single dose, iv) was administered immediately after reperfusion. Anti-irisin (4 mg/kg, Abcam) blocking antibodies were administered at 24 h before gut IR. Four hours after reperfusion, mice were sacrificed, and tissue samples were collected. A, Serum irisin levels; (B,C) Western blot analysis of irisin expression; (D,E) immunofluorescence staining of irisin (green) and the corresponding nuclear counterstaining (blue) in gut tissues; (F) gut injury score; (G) haematoxylin and eosin (H&E) staining; (H) water content of gut; (I) serum FITC-dextran levels; (J,K) colony-forming units (CFUs) from mesenteric lymph node (MLN) and lung tissues; (L,M) serum levels of LDH and lactate; and (N,O) serum TNF- $\alpha$  and C1RP levels.  $n = 6$  per group, mean  $\pm$  SEM, \* $P < .05$  vs the sham group, # $P < .05$  vs the gut IR group Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/31701659>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Irisin decreases the oxidative stress, calcium influx and ER stress after gut IR. Irisin (250  $\mu\text{g}/\text{kg}$  in 0.5 mL saline, a single dose, iv) was administered immediately after reperfusion. Anti-irisin (4 mg/kg, Abcam, USA) blocking antibody was administered at 24 h before gut IR. Four hours after reperfusion, mice were sacrificed, and tissue samples were collected. A,B, DHE fluorescence staining of gut tissues; (C-G) levels of gut malonaldehyde (MDA), xanthine oxidase (XO), 4-hydroxynonenal (4-HNE), superoxide dismutase (SOD) and glutathione peroxidase activity (GSH-PX), respectively;  $n = 6$  per group, mean  $\pm$  SEM, \* $P < .05$  vs the sham group, # $P < .05$  vs the gut IR group. Caco-2 cells were exposed to hypoxia for 90 min, and 10 nmol/L irisin was added at the beginning of reoxygenation. (H,I) DHE fluorescence staining; (J,K) Fluo-4 AM staining of  $\text{Ca}^{2+}$ ; (L,M) Western blot analysis of IRE1 and CHOP expression at 4 h after reoxygenation in Caco-2 cells;  $n = 3$  per group, mean  $\pm$  SEM, \* $P < .05$  vs the sham group, # $P < .05$  vs the H/R group Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/31701659>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Irisin protects against gut IR injury via binding to integrin  $\alpha\beta5$  receptor in enterocyte. Caco2 cells were exposed to hypoxia for 90 min, and 10 nmol/L irisin and 20  $\mu\text{mol/L}$  cilengitide trifluoroacetate were added at the beginning of reoxygenation. A, Immunofluorescence staining of irisin (green), integrin  $\alpha\beta5$  (red) and the corresponding nuclear counterstaining (blue) in Caco2 cells at 4 h after reoxygenation in Caco2 cells. Irisin (250  $\mu\text{g/kg}$ , iv) and cilengitide trifluoroacetate (20 mg/kg, iv) were administered immediately after reperfusion. Four hours after reperfusion, mice were sacrificed, and tissue samples were collected. (B) COIP of irisin and integrin  $\alpha\beta5$ ; (C) serum FITC-dextran levels; (D) colony forming units (CFUs) from mesenteric lymph node (MLN) tissues; (E) haematoxylin and eosin (H&E) staining; (F) gut injury score;  $n = 6$  per group, mean  $\pm$  SEM, \* $P < .05$  vs the gut IR group, # $P < .05$  vs the gut IR + irisin group. (G,H) Western blot analysis of claudin1 and occludin expression in Caco2 cells; and (I,J) immunofluorescence staining of JAM1 and ZO1 and the corresponding nuclear counterstaining (blue). \* in the figure represents intercellular gap.  $n = 3$  per group, mean  $\pm$  SEM, \* $P < .05$  vs the sham group, # $P < .05$  vs the H/R group Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/31701659>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Maha Saber, Amanda D. Rice, Immaculate Christie, Rebecca G. Roberts, Kenneth S. Knox, Peter Nakaji, Rachel K. Rowe, Ting Wang, Jonathan Lifshitz Remote ischemic conditioning reduced acute lung injury after traumatic brain injury in the mouse Shock (Augusta, Ga.) 2021-02-01 [PMID: 32769821] (Immunohistochemistry, Human)

Kirat D, Hamada M, Moustafa A, Miyasho T Irisin/FNDC5: A participant in camel metabolism Saudi Journal of Biological Sciences 2021-01-01 [PMID: 33424357] (IHC-P, Camel)

Kirbas A, Karakus E, Ozkanlar S et al. FNDC5 Gene Expression in the Smooth Muscles of Dromedary Camels (Camelusdromedarius) Int J Vet Sci 2020-01-01

Bi J, Zhang J, Ren Y et al. Irisin reverses intestinal epithelial barrier dysfunction during intestinal injury via binding to the integrin alpha V beta 5 receptor J. Cell. Mol. Med. 2019-11-07 [PMID: 31701659] (IF/IHC, Human)

## Procedures

### Western Blot protocol for Irisin/FNDC5 Antibody (NBP2-59680)

Irisin/FNDC5 Antibody:

1. Perform SDS-PAGE on samples to be analyzed, loading 25 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 anti-Irisin/FNDC5 primary antibody 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 the diluted 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%.

### Immunocytochemistry/Immunofluorescence protocol for Irisin/FNDC5 Antibody (NBP2-59680)

Irisin/FNDC5 Antibody:

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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### **Products Related to NBP2-59680**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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### **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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