Product Manual

Irisin ELISA Kit

Catalog Number

MET-5089 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Irisin is the soluble cleavage product of Fibronectin type III domain-containing protein 5 (FNDC5) and is named after Iris, the messenger of the Gods found in Greek mythology. The FNDC5 gene encodes a single-pass type I membrane pro-hormone (human, 212 amino acids; mouse and rat, 209 amino acids). Irisin is synthesized like the production of other hormones and hormone-like polypeptides such as epidermal growth factor and TGF alpha. After the N-terminal signal peptide of FNDC5 is excised, Irisin is proteolytically cleaved from the C-terminal moiety, glycosylated, and released as a hormone of 112 amino acids (in human, amino acids 32-143 of the full-length protein; in mouse and rat, amino acids 29-140) that contains much of the FNIII repeat region (Figure 1). In mice, exercise causes increased muscle expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1alpha), which is involved in adaptive responses to exercise. Upregulation of PGC-1alpha causes expression of FNDC5 which is processed to Irisin. Since Irisin is produced through a mechanism started by muscle activity, Irisin is considered to be a myokine. Demonstration that FNDC5 causes an increase in thermogenin expression in fat cells, that overexpression of FNDC5 in mouse liver blocks diet-induced weight gain, and FNDC5 levels of mRNA are increased in human muscle post-exercise, have led researchers to propose that Irisin stimulates white fat to brown fat conversion which would suggest it is a health promoting hormone in humans.

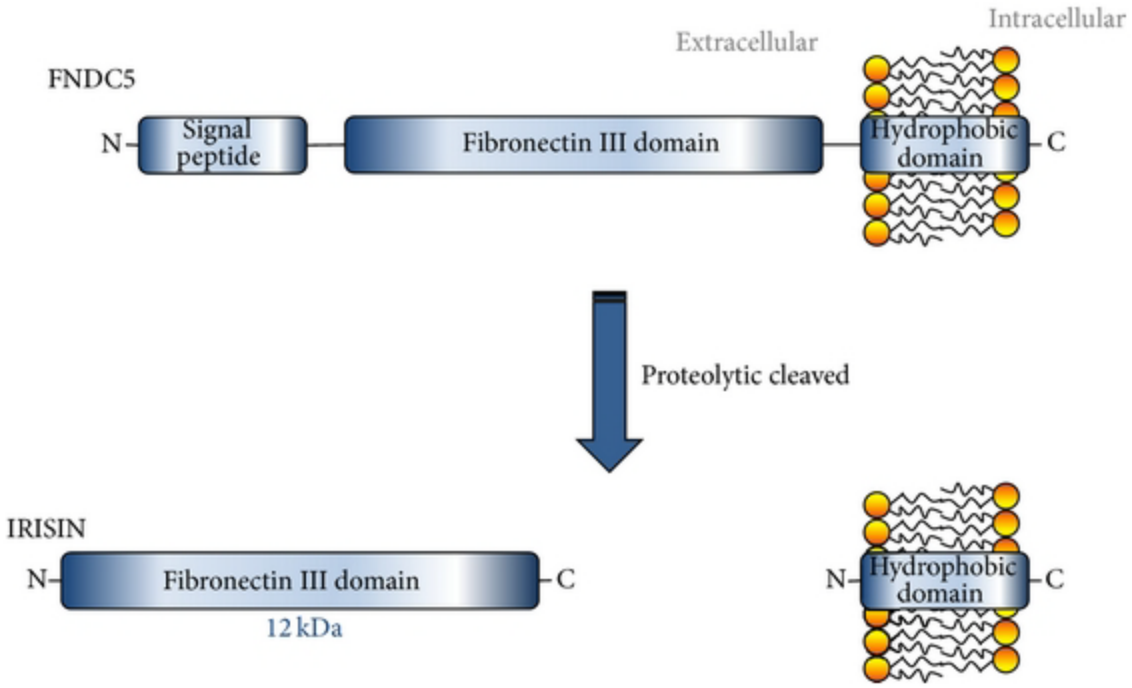

Figure 1: Structure of FNDC5/Irisin

Cell Biolabs' Irisin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of Irisin in plasma, serum, urine, cell or tissue lysate samples. The kit has a detection sensitivity limit of 6.25 ng/mL Irisin. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. MET-5011: Lipid Droplet Isolation Kit
2. MET-5022: Glycogen Assay Kit (Colorimetric)
3. MET-5023: Glycogen Assay Kit (Fluorometric)
4. MET-5052: Adiponectin ELISA Kit
5. MET-5063: Insulin ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Irisin Antibody Coated Plate (Part No. 50891B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Irisin Antibody (1000X) (Part No. 50892C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Irisin Standard (Part No. 50893D): One 50 µL vial of 40 µg/mL recombinant Irisin expressed in CHO cells.

Materials Not Supplied

1. Plasma, serum, cell or tissue lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Irisin Standard at -80ºC and store the Biotinylated Anti-Irisin Antibody (1000X) at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

- Biotinylated Anti-Irisin Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Irisin Antibody or the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human Irisin Standard

Prepare a dilution series of human Irisin standards in the concentration range of 0 to 400 ng/mL into Assay Diluent (Table 1).

Standard Tubes	40 µg/mL Irisin Standard (µL)	Assay Diluent (µL)	Irisin (ng/mL)
1	8	792	400
2	400 of Tube #1	400	200
3	400 of Tube #2	400	100
4	400 of Tube #3	400	50
5	400 of Tube #4	400	25
6	400 of Tube #5	400	12.5
7	400 of Tube #6	400	6.25
8	0	400	0

Table 1. Preparation of Irisin Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Urine: Harvest urine and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of Irisin unknown sample or standard to the Anti-Human Irisin Antibody Coated Plate. Each Irisin unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 2 hours at 37°C or 4°C overnight.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Irisin Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Irisin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

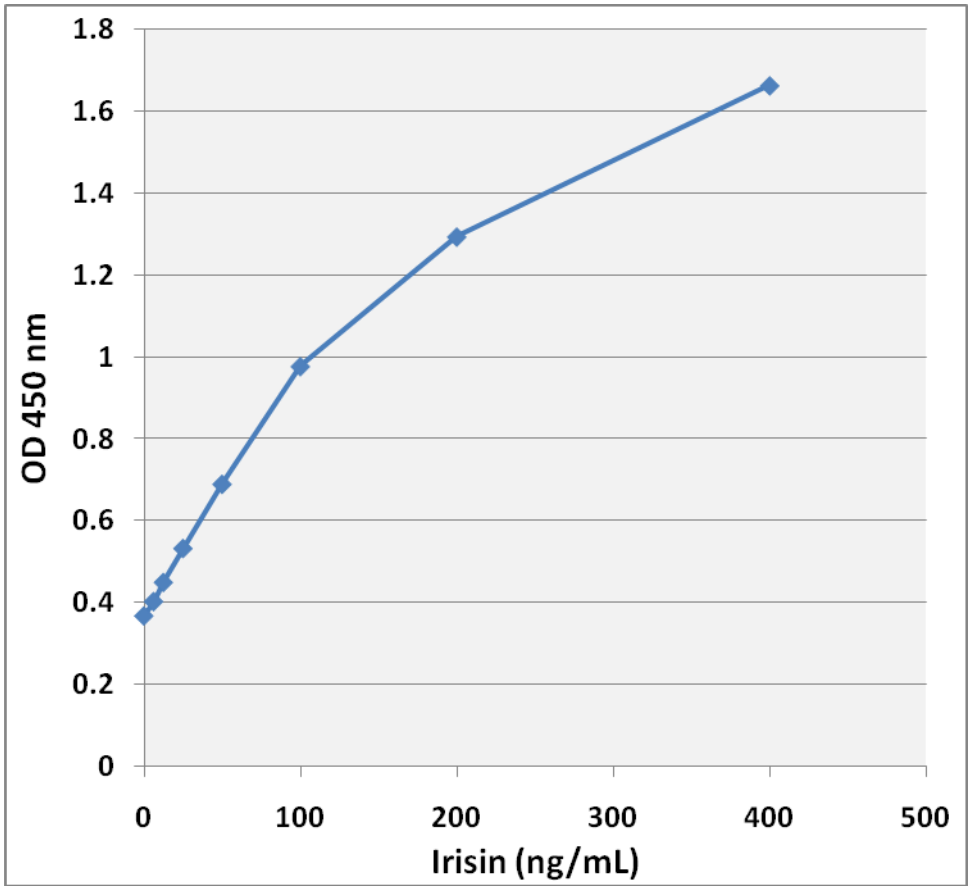

Figure 2: Irisin Standard Curve.

References

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2. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, and Spiegelman BM (2012). *Nature*. **481**: 463–8
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4. Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui T, Yang LJ, and Tang D (2014). *Diabetes*. **63**: 514–25.
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Warranty

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