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MonELISA® Series
Irisin (FNDC5) Assay

Catalog Number M036085

For the quantitative determination of Irisin (FNDC5) in serum, plasma, cell culture supernatant, or tissue homogenate

For research use only.

INTRODUCTION

Irisin is one of the recently discovered and isolated hormones containing 112 amino acids. It is secreted from muscles in response to exercise and may mediate some beneficial effects of exercise in humans, such as weight loss and thermoregulation

The MonELISA® Irisin is a colorimetric based sandwich immunoassay utilizing a polyclonal antibody to Irisin bound on the surface of microwells as the capture antibody and a biotinylated polyclonal antibody to irisin as the detection antibody. When antigen is added to the well it is bound by the immobilized antibody. The biotinylated antibody then binds to the antigen forming an antigen-antibody complex. Streptavidin-Peroxidase in the presence of an enzyme substrate quantifies analyte bound. Color development is directly proportional to the antigen concentration in the sample.

KIT COMPONENTS

Irisin specific Microplate - The plate contains 12 x 8 strips coated with polyclonal antibody to Irisin. The strips are ready to use.

Irisin Standard - 1 vial of Irisin in a protein buffer, 30 ng/ml

Conjugate Concentrate - 1 vial of a 100-fold concentrated biotinylated anti-Irisin antibody in a stabilizing buffer diluted in buffer provided in Ancillary Reagent Kit, Catalog Number M036080.

HRP Streptavidin Concentrate - 1 vial of a 100-fold concentrated streptavidin-HRP diluted in buffer provided in Ancillary Reagent Kit, Catalog Number M036080

THESE ASSAY COMPONENTS ARE TO BE USED WITH
5-PLATE ANCILLARY REAGENT KIT, CATALOG NUMBER M036080

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Measuring cylinders
- Distilled or deionized water
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm

PRECAUTIONS

Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

The Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

- For research use only. Not for internal or external use in humans or animals.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (22 - 25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done manually, ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

REAGENT PREPARATION

Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge before use. All diluents required are provided in the Ancillary Reagent Kit Catalog number M036080.

Wash Buffer (1X) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL of deionized water to prepare 500 mL of Wash Buffer (1X). Store for up to 30 days at 2 - 8 °C.

Conjugate (1X) - Dilute 120 µL of Conjugate Concentrate into 12 mL of Conjugate diluent. Discard after use.

Streptavidin-HRP (1X) - **Prepare within 30 minutes of use and keep protected from light**. For a complete plate, add 120 µL Streptavidin-HRP Concentrate to 11.88 mL of HRP Diluent. Prepare fresh Streptavidin-HRP (1X) for each assay. If running less than a full plate, prepare only the amount needed.

Standards -

Irisin Serial Standard Dilution:

- Label 7 standard tubes.
- Pipette 400 µL Assay Diluent and 100 µL of the 30ng/ml standard to yield the 6000 pg/mL standard in the first tube.
- Add 250 µL Assay Diluent into the remaining 6 tubes. Perform a 6-step serial dilution 1:2 starting with the 6000 pg/ml standard.
- The 6000 ng/mL standard serves as the high standard and Assay Diluent serves as the zero (0 pg/mL) standard. The standards will range from 93.75 pg/ml to 6000 pg/ml.

ASSAY PROTOCOL

It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge before use.

Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Add 100 µL of Standard or diluted sample in duplicate to each well. Cover with the plate sealer provided and incubate for 1 hour at 37 °C.
4. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 100 µL Conjugate (1X) to each well. Cover with the plate sealer provided and incubate for 1 hour at 37 °C.
6. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 100 µL of diluted Streptavidin-HRP to each well. Incubate for 30 minutes at 37 °C. **Protect from light.**
8. Aspirate and wash the wells 4 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
9. Add 100 µL Substrate to each well and incubate for 10 minutes at 37 °C. **Protect from light.**
10. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently tap the side of the plate to ensure thorough mixing.
11. Read the plate at 450 nm.

SUMMARY

Prepare reagents and samples as previously described.

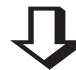
Pipette 100 µL Standard or diluted sample in duplicate to each well.
Cover with plate sealer and incubate 1 hr. at 37 °C.

Aspirate and wash 4 times.

Add 100 µL of Conjugate (1X) to each well.
Cover with plate sealer and incubate 1 hr. at 37 °C.

Aspirate and wash 4 times.

Add 100 µL of diluted Streptavidin-HRP to each well. Incubate 30 min. at 37 °C. Protect from Light.



Aspirate and wash 4 times.

Add 100 µL of Substrate to each well. Incubate 10 min. at 37 °C. Protect from Light.

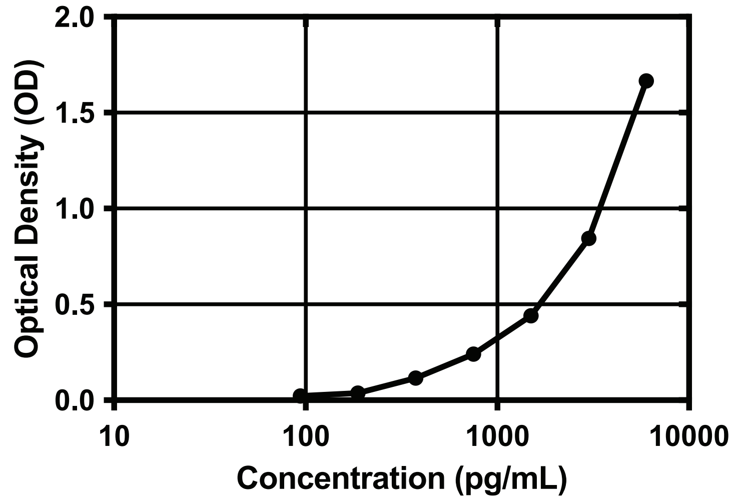
Add 100 µL of Stop Solution to each well. Read at 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using 4-parameter logistic (4-PL) curve fit.

This standard curve is provided for demonstration only. A standard curve should be generated with each set of samples assayed.

Range: 93.75-6000 pg/ml



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the assay is 40 pg/ml

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 15-20 times on one plate.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 15 successive assays with multiple users.

	Intra-assay Precision			Inter-assay Precision		
Control	1	2	3	1	2	3
Mean (ng/mL)	171.95	524.47	1917.93	164.03	513.29	1942.25
Standard Deviation	29.44	54.30	99.29	30.82	61.92	86.67
CV (%)	17.12	10.36	5.18	18.79	12.06	4.46

TROUBLESHOOTING

Problem	Recommendation
Low Absorbance	· Check reagents for proper storage. · Check expiration date. · Check preparation of reagents. · Check incubation times and temperature. · Check reader wavelength.
High Absorbance/high zero standard value	· Check preparation of reagents. · Check incubation times and temperature. · Equilibrate ELISA reagents to room temperature (22 - 25 °C). · Ensure that every well of the ELISA plate is filled completely and emptied at every wash step. · Check that plates are blotted on tissue paper after washing.
Flat curve/poor reproducibility	· Check reagents for proper storage. · Check expiration date. · Check preparation of working standards. · Check incubation times and temperatures. · Use separate reservoirs for pipetting different solutions with multichannelled pipettes. Always use new pipette tips. · Check pipette calibration. · Ensure efficient washing procedure.

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