Irisin ELISA Kit

Competitive Enzyme Immunoassay for the quantification of Irisin in Human, Mouse, Rat, Canine plasma (EDTA) and serum samples

Catalog number: ARG81195

Package: 96 wells

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INTRODUCTION

This gene encodes a secreted protein that is released from muscle cells during exercise. The encoded protein may participate in the development of brown fat. Translation of the precursor protein initiates at a non-AUG start codon at a position that is conserved as an AUG start codon in other organisms. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jun 2013]

Irisin: mediates beneficial effects of muscular exercise. Induces browning of white adipose tissue by stimulating UCP1 expression, at least in part, via the nuclear receptor PPARA (By similarity) [UniProt]

PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification of Irisin in Serum and plasma samples

This assay employs the competitive enzyme immunoassay technique. A secondary antibody has been pre-coated onto a microtiter plate and non-specific binding sites were blocked. Fc regions of primary antibodies specific for target peptides can bind to the secondary antibodies on microtiter plate. The Fab regions of primary antibodies are competitively bound by biotinylated peptide and targeted peptides in samples or standards. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The reaction is monitored by a color change which is readable at OD of 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared

with known standards. The intensity of color development is inversely proportional to the amount of Irisin in the samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Secondary antibody coated microplate	12 x 8 wells	4°C
20X assay buffer concentrate	50 ml	4°C
Primary antibody	1 vial	4°C
Biotinylated peptide	1 vial	4°C
Standard	1 vial	4°C
1000X Streptavidin-HRP conjugate	30 µl	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution (2N HCl)	15 ml (Ready-to-use)	4°C
Positive control (Accept. Range: 4.0 -8.0 ng/ml)	2 vials	4°C
Plate sealer	3 pieces	Room Temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution reservoir
- Aprotinin (0.6TIU/ml of blood) (optional)
- Deionized or distilled water

- Orbital microplate shaker capable of 300-400rpm (Recommended)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lyophilized material is at the bottom of the tube.
- If crystals are observed in the 20X Assay buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that all solutions be used as soon as possible after reconstitution.
- Unused microplate strips should be placed back in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can

accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.

- For optimal results, an orbital plate shaker capable of 300-400 rpm is recommended for all incubations.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Blood collection- Collect blood samples into tubes containing EDTA. Gently rock the tubes several times immediately to prevent coagulation. Transfer the blood from tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml blood) and gently rock for a few times to inhibit activity of proteinases. Centrifuge the blood at 1600 x g for 15 minutes at 4°C and collect plasma. Plasma can be kept at- 80°C up to 1 month.

Serum- Use a serum separator tube (SST) (add Aprotinin as enzyme inhibitor is recommended) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1600 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at -80°C up to 1 month. Avoid repeated freeze-thaw cycles.

Note: We recommended add Aprotinin (enzyme inhibitor) for **ALL** sample collection to prevent sample degradation. 0.6 TIU or 100 µl of Aprotinin per mL of sample solution.

REAGENT PREPARATION

- **1X Assay Buffer**: Dilute **20X** Assay Buffer into **distilled water** to yield 1X Assay Buffer (e.g. 50ml of 20X Assay Buffer + 950ml distilled water). Keep 1X Assay Buffer at 4°C. If crystals appear in 20X Assay Buffer, warm the buffer in warm water bath for 30 minutes or until crystals disappear. Mix well before use.
- **Primary antibody**: Reconstitute the Primary antibody vial with **5 ml of 1X assay buffer**. Allow it to sit for 5 minutes to completely dissolve, mix well and keep rehydrated solution at 4°C before use. Store the reconstituted antibody at 4°C up to a week. For long-term storage, aliquot & store at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles. It is recommended that antibody should only be frozen-thawed once.
- **Biotinylated peptide**: Reconstitute the Biotinylated peptide vial with **5 ml of 1X assay buffer**. Allow it to sit for 5 minutes to completely dissolve, mix well and keep rehydrated solution at 4°C before use. Store the reconstituted peptide at 4°C up to a week. For long-term storage, aliquot & store at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles. It is recommended that peptide should only be frozen-thawed once.
- **1X Streptavidin-HRP conjugate:** 1X Streptavidin-HRP conjugate working solution should be prepared freshly before use. Centrifuge 1000X Streptavidin-HRP conjugate briefly (3,000-5,000 rpm for 5 seconds) and add **12µl of Streptavidin-HRP conjugate** to **12ml of 1X assay buffer** to make a 1X Streptavidin-HRP working solution. Vortex thoroughly.

- **Positive control**: Centrifuge the Positive control before opening. Reconstitute the Positive control vial with **200 µl** of **1X assay buffer**. Allow to sit for at least 5 minutes and mix well to dissolve completely before use. Keep rehydrated solution at 4°C and use as soon as possible.
- **Sample:** Serum and plasma samples are recommended to **dilute with equal volume of 1X assay buffer** before assay (dilution factor =2). It is recommended each laboratory to do pre-test to determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve. Samples can be diluted with 1x assay buffer if needed. For the calculation of the concentrations this dilution factor has to be taken into account.
- **Standard**: Reconstitute the Standard vial with **1 ml** of **1X assay buffer**. Vortex it. The concentration of this stock solution is **1000 ng/ml**. Allow to sit for 10 minutes at room temperature (20-23°C) to dissolve completely. Mix well and spin down before use. Dilute standard solutions according to the table below and make serial dilutions of **1000 ng/ml**, **100 ng/ml**, **10 ng/ml**, **1 ng/ml** and **0.1 ng/ml**.

Standard No.	Standard Conc. (ng/ml)	1X Assay Buffer	Standard volume
S1 (Stock)	1000	0 µl	1000 µl
S2	100	900 µl	100 µl of S1
S3	10	900 µl	100 µl of S2
S4	1	900 µl	100 µl of S3
S5	0.1	900 µl	100 µl of S4
S0 (Total binding)	0	150	0

ASSAY PROCEDURE

Note: All materials should be equilibrated to room temperature (20-23°C) before use or opening. It is recommended that the solutions be used as soon as possible after rehydration. Standards, Samples and controls should be assayed in duplicates. Do not allow moisture to enter the wells.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **1X assay buffer** as Total Binding (S0, zero standard). Keep another Two Empty Wells as Blank.
3. Add **50 µl** of **prediluted peptide standards** (add from S5 to S1), **50 µl positive controls** or **50 µl samples** into corresponding wells. It is advisable to assay each condition in duplicates.
4. Add **25 µl** of **primary antibody** into each well **except the Blank wells**.
5. Add **25 µl** of **Biotinylated peptide** into each well **except the Blank wells**. It is not recommended to use a multi-channel pipette to load the primary antibody and biotinylated peptide.
6. Seal the microtiter plate with Plate sealer. Incubate for **2 hours at room temperature (20-23°C)**. Orbital shaking at 300-400 rpm with a microplate shaker is recommended.
7. Centrifuge Streptavidin-HRP vial to spin down the solution in the vial and pipette 12 µl of Streptavidin-HRP concentrate into 12ml of 1X Assay buffer to make a Streptavidin-HRP working solution. Vortex thoroughly. Prepare freshly.
8. Remove sealer from plate.

9. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Assay Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Assay Buffer by aspirating, decanting or blotting against clean paper towels.
10. Add **100 µl** of **diluted (1X) Streptavidin-HRP solution** into **each well**.
11. Reseal the plate with sealer. Incubate for **1 hour at room temperature (20-23°C)**. Orbital shaking at 300-400 rpm with a microplate shaker is recommended.
12. Remove sealer from plate.
13. Wash as according to step 9.
14. Add **100 µl** of **TMB substrate solution** into **each well**.
15. Reseal the plate with sealer. Incubate for **1 hour at RT in dark.** Orbital shaking at 300-400 rpm with a microplate shaker is recommended.
16. Remove sealer from plate. (**DO NOT** wash or discard the contents of the wells)
17. Add **100 µl** of **STOP solution (2N HCl)** into **each wells** to stop the reaction. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
18. Read the OD with a microplate reader at **450 nm** immediately. It is recommended that the wells be read within 20 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

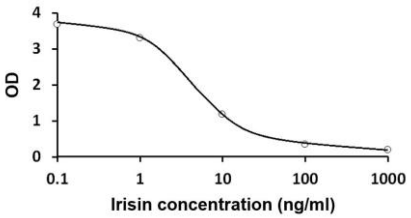
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Standard Range: 0.1- 1000 ng/ml

Linear Range: 2.62 – 23.3 ng/ml

Sensitivity: 2.62 ng/ml

Cross Reactivity

The cross-reactivity with the following factors were as below:

Irisin, Recombinant (Human, Mouse, Rat, Dog): 100%

Irisin (aa. 1-112) (Human, Mouse, Rat, Dog): 100%

FNDC5, isoform 4 recombinant (Human, Mouse, Rat): 9%

Irisin precursor, C-terminal 48-mer: Human FNDC5 (aa. 165-212); Mouse, Rat FNDC5 (aa. 162-209): 0%

Intra-assay and Inter-assay precision

Intra-assay variation: <10%

Inter-assay variation: <15%