# Irisin ELISA Kit

# (For Human, Mouse, Rat, Canine)

Competitive Enzyme Immunoassay for the quantification of Irisin in Plasma and Serum samples

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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 |
|---|---|---|
| Microtiter Plate | 12 x 8 wells | 4°C |
| 20X assay buffer concentrate | 50 ml | 4°C |
| Acetate Plate sealer (APS) | 3 pieces | 4°C |
| Primary antibody (Rabbit anti-peptide IgG) | 1 vial | 4°C |
| Standard peptide | 1 vial | 4°C |
| Biotinylated peptide | 1 vial | 4°C |
| Streptavidin-HRP | 30 µl | 4°C |
| Positive control | 2 vials | 4°C |
| Substrate Solution (TMB) | 12 ml | 4°C, ready for use |
| 2N HCl | 15 ml | 4°C, ready for use |

## 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.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Clooect serum and add aprotinin (0.6 TIU/ml blood) and gently rock for a few times to inhibit activity of proteinases. Assay immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma on ice using EDTA as an anticoagulant. 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. Avoid repeated freeze-thaw cycles.

** In order to reduce background, peptide extraction of samples is highly

recommended **

Peptide extraction –

1. Mix an equal amount of Binding Buffer (1% trifluoroacetic acid (TFA), HPLC grade is recommended) with the plasma or serum samples and vortex the mixture. Centrifuge at 6,000 -17,000 × g for 20 minutes at 4°C. Collect the supernatant.
2. Slowly equilibrate a C18 column (SEP-COLUMN containing 200 mg of C18) by washing the C18 column with 1 ml Elution Buffer (60% acetonitrile, 1% TFA, and 39% distilled water, HPLC grade of acetonitrile and TFA are recommended).
3. Wash the C18 column with 3 ml of Binding Buffer three times.
4. Load the sample/Binding Buffer mixture solution from step 1 into the washed C18 column from step 3.
5. Wash the column slowly with Binding Buffer (3 ml, twice) and discard the wash.
6. Elute the peptide slowly with Elution Buffer (3 ml, once) and collect eluant into a polystyrene tube.
7. Evaporate eluant to dryness in a centrifugal concentrator or by a suitable substitute method to dryness the eluant. (Freeze-dry the resulting water/TFA solution to dryness)
8. Keep the dried extract at -20°C and perform the assay as soon as possible.
9. For normal subject extracted from 1 ml original plasma/serum, use 125 µl 1x assay buffer to reconstitute the dried extract. Aliquot 50 µl into two designated assay wells (25 µl is left over). The concentration factor in this case is 8. (1 ml / 125 ul = 8). The original plasma peptide level is 1/8 of the

level of final extracted plasma.

e.g. If the level of the final extracted plasma is 100 pg/ml, then the total level of peptide in the original plasma = (100 pg/ml) / 8 = 12.5 pg/ml. After performing assay, if the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the samples accordingly.

## 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 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.
- **Biotinylated peptide**: Reconstitute the Biotinylated peptide vial with 5 ml 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.
- **Positive control**: 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:** 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 peptide**: Reconstitute the Standard peptide 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.

| Tube No. | Standard volume | 1X Assay Buffer | Concentrations (ng/ml) |
|---|---|---|---|
| Stock | 1000 µl | 0 µl | 1000 |
| 1 | 100 µl of stock | 900 µl | 100 |
| 2 | 100 µl of Tube 1 | 900 µl | 10 |
| 3 | 100 µl of Tube 2 | 900 µl | 1 |
| 4 | 100 µl of Tube 3 | 900 µl | 0.1 |

## 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. Samples and controls should be assayed in duplicates. Unused microplate strips should be placed in the foil pouch with a dessicant and stored at 4°C. Do not allow moisture to enter the wells.

1. Add 50 µl of 1X assay buffer as Total Binding. Keep another Two Empty Wells as Blank.

2. Add 50 µl of peptide standards (1000 ng/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml and 0.1 ng/ml), 50 µl positive controls or 50 µl samples into corresponding wells. It is advisable to assay each condition in duplicates.
3. Add 25 µl of primary antibody into each well except the Blank wells.
4. 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.
5. Seal the microtiter plate with acetate plate sealer (APS). Incubate for 2 hours at room temperature (20-23°C). Orbital shaking at 300-400 rpm with a microplate shaker is recommended.
6. Centrifuge Streptavidin-HRP vial to spin down the solution in the vial and pipette 12 µl of Streptavidin-HRP into 12ml of 1X Assay buffer. Vortex thoroughly. Prepare fresh.
7. Remove sealer from plate.
8. 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 Wash Buffer by aspirating, decanting or blotting against clean paper towels.
9. Add 100 µl diluted Streptavidin-HRP solution into each well.
10. 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.
11. Remove sealer from plate.

12. Wash as according to step 8.
13. Add 100 µl TMB substrate solution into each well.
14. Reseal the plate with sealer. Incubate for 1 hour at RT. Orbital shaking at 300-400 rpm with a microplate shaker is recommended. (Protect from light)
15. Remove sealer from plate. Add 100 µl 2N HCl into all wells to stop the reaction, gently tap the plate to ensure thorough mixing. The color in the well should change from blue to yellow. Go to the next step within 20 minutes.
16. Read the OD with a microplate reader at 450 nm immediately.

## CALCULATION OF RESULTS

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

2. Using semi-log 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.

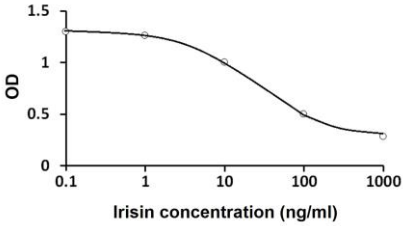
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. 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: 6.8- 96.1 ng/ml

Sensitivity: 6.8 ng/ml

### Cross Reactivity

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

Irisin (Human, Rat, Mouse): 100%

Irisin (aa. 42-112) (Human, Rat, Mouse): 100%

Irisin precursor, C-terminal 48mer/ FNDC5 (aa. 149-196) (Human)/ FNDC5 (aa. 162-209) (Rat, Mouse): 0%

Irisin (aa. 42-95) (Human, Rat, Mouse): 0%

## Intra-assay and Inter-assay precision

Intra-assay variation: <10%

Inter-assay variation: <15%

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***For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.***

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