Version 07-02/25

User's Manual

Recombinant Human/Rat/Mouse/Canine Irisin ELISA Kit

REF **DEIA-WZ311**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is designed to measure the concentration of a specific peptide and its related peptides based on the principle of a "competitive" enzyme immunoassay.

General Description

Irisin is one of the most recently discovered and isolated hormones, derived from mouse skeletal muscle in 2012. Irisin is secreted from muscles in response to exercise and may mediate some beneficial effects of exercise in humans, such as weight loss and thermoregulation. Irisin consists of 112 aa residues. Irisin is a cleavage protein of fibronectin type III domain 5 (FNDC5). Mouse, rat, and human irisin are 100% identical; the rates of similarity are 85% for insulin, 90% for glucagon, and 83% for leptin. Irisin is expected to be a potential therapeutic agent for the treatment of obesity and its related conditions. FNDC5 converts to irisin after exercise. An increase of irisin causes the browning of human and mouse white adipose tissues.

Principles of Testing

The plate in this kit is pre-coated with a secondary antibody, whose nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody. This primary antibody's Fab fragment will then be competitively bound by both the biotinylated peptide and the targeted peptide in either the standard peptide solution or the unknown sample. The biotinylated peptide interacts with streptavidin horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The intensity of the resulting yellow color is directly proportional to the amount of biotinylated peptide-SA-HRP complex, but inversely proportional to the amount of the targeted peptide (in either the standard peptide solution or the unknown sample). This is due to competition between the biotinylated peptide and the target peptide for binding with the primary antibody. A standard curve can be established by plotting the measured O.D. as a function of the various known standard peptide concentrations. Unknown peptide concentration in samples can then be determined via extrapolation based on this standard curve.

Reagents And Materials Provided

1. EIA assay buffer concentrate (20x, 50ml)
2. Pre-coated EIA plate (96 wells, 1 plate)
3. Acetate plate sealer (APS) (3 pieces)
4. Primary antibody (1 vial)
5. Standard peptide (1 vial)
6. Biotinylated peptide (1 vial)
7. Positive control (2 vials)
8. Streptavidin-horseradish peroxidase (SA-HRP) concentrate (30µl)
9. Substrate solution (12ml)
10. 2N HCl (Stop solution) (15ml)

Materials Required But Not Supplied

1. Microtiter plate reader (450nm) (required)
2. Micropipette with disposable pipette tips (required)
3. Absorbent material for blotting (required)
4. Vortex (required)
5. Curve-fitting software capable of 4 parameter logistics (recommended)
6. Orbital plate shaker (300-400rpm) (recommended)
7. Microtiter plate washer (recommended)
8. Multi-channel pipette (50-100µl) (recommended)
9. Solution reservoir (recommended)
10. Centrifuge (optional)
11. EDTA Lavender Vacutainer blood collection tubes (optional)
12. Aprotinin (30 TIU) (optional)

Storage

Store the kit at 4°C upon receipt. Do not freeze.

Specimen Collection And Preparation

General Tissue Preparation:

1. Boil tissue in 75% HoAc (Acetic Acid) for 20 minutes at 100°C.
2. Homogenize tissue in lysis buffer, usually with a low pH.
3. Centrifuge the tissue homogenate at 12,000rpm for 20 to 30 minutes at 4°C.
4. For peptide extraction from sample, take 1ml of supernatant and combine with 1ml of Buffer A to acidify sample. Centrifuge at 6,000 to 17,000 x g for 20 minutes and collect the supernatant. This will be loaded onto the C-18 SEP-COLUMN. Performing the centrifugation on ice helps to inhibit peptidases.

Note: If a separate protein assay is required, designate and remove an aliquot before addition of Buffer A. This buffer contains materials which may interfere with protein analysis.

5. Equilibrate a SEP-COLUMN containing 200mg of C18. Wash with 1ml of Buffer B once, followed by 3ml of Buffer A three (3) times.
6. Load the acidified sample (plasma, serum, tissue, etc.) solution onto the pre-equilibrated C-18 SEP-COLUMN.
7. Slowly wash the column with 3ml of Buffer A twice and discard the wash.
8. Elute the peptide slowly with 3ml of Buffer B once and collect the eluant into a polystyrene tube.

Note: Ensure there is a constant flow for all solutions during the extraction procedure. For optimal sample processing and recovery, do not allow air bubbles to enter the C-18 matrix.

9. Evaporate eluant to dryness in a centrifugal concentrator or by a suitable substitute method.

Note: A combination of a centrifugal concentrator (i.e. Speedvac) and a lyophilizer (freeze-dryer) produces the best results. First, use a centrifugal concentrator to dry the sample for approximately

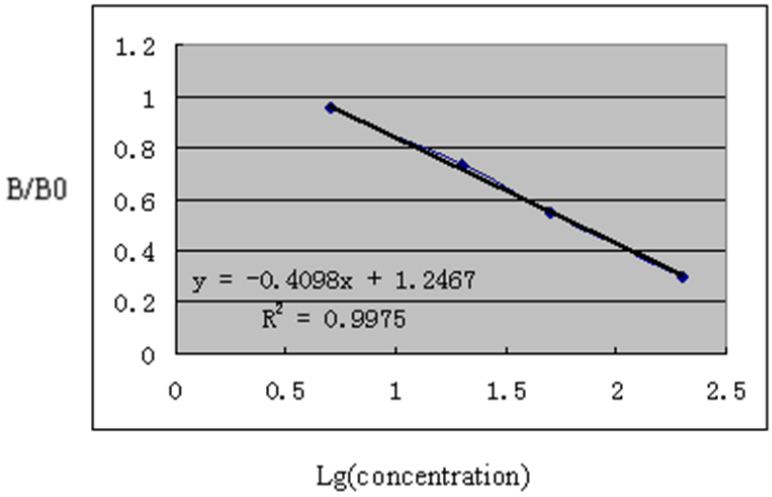
15 minutes, removing the organic layer. Snap-freeze the remaining sample and freeze-dry overnight using a lyophilizer. If a centrifugal concentrator is not accessible, freeze-drying overnight using a lyophilizer will be sufficient.

10. Keep the dried extract at -20°C and perform the assay as soon as possible. Use the 1x assay buffer to reconstitute the dried extract to the desired concentration. If the peptide value does not fall within the range of detection, dilute or concentrate the sample accordingly.

Note: For example, if 1ml of plasma was extracted, dried, and then reconstituted in 250µl of 1x assay buffer, then the original sample would have now have undergone a 4x concentration

Plate Preparation

Reagent Preparation



Assay Procedure

Note: The kit and all its components should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. 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.

1. Dilute the 20x EIA assay buffer concentrate with 950ml of distilled water. Mix thoroughly before use. This will be the 1x assay buffer solution used to dilute or reconstitute all other samples and reagents during the assay.

Note: If crystals appear in the 20x assay buffer, the bottle can be placed in a warm water bath for approximately 30 minutes or until no crystals are visible.

2. Reconstitute the standard peptide in 1ml of the 1x assay buffer and vortex thoroughly. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. This will be the standard stock solution.

Note: Vortex immediately before use.

3. Reconstitute the primary antibody in 5ml of 1x assay buffer and vortex thoroughly. Allow the solution to sit for at least 5 minutes at room temperature to completely dissolve.
4. Reconstitute the biotinylated peptide in 5ml of 1x assay buffer and vortex thoroughly. Allow them solution to sit for at least 5 minutes at room temperature to completely dissolve.
5. Reconstitute the positive control in 200µl of 1x assay buffer and vortex thoroughly. Allow the solution to sit for at least 5 minutes at room temperature to completely dissolve.
6. Prepare the standard dilutions from the rehydrated standard peptide as shown on Section Reagent Preparation. Vortex the tube thoroughly after each serial dilution.
7. Leave wells A1 and A2 on microplate empty as blanks.
8. Add 50µl of 1x assay buffer into wells B1 and B2. These will represent total binding (of the biotinylated peptide).
9. Add 50µl of the least concentrated peptide standard solution (#4) to wells C1 and C2. Next, add peptide standard #3 into wells D1 and D2, and so forth, continuing in the opposite order of the standard dilution to G1 and G2.

Note: Standard peptides should always be assayed in duplicate.

10. Add 50µl of rehydrated positive control into wells H-1 and H-2.

Note: Positive controls should always be assayed in duplicate.

11. Add 50µl of any unknown/prepared samples into their designated wells, again in duplicate.

Note: Each laboratory must determine the appropriate dilution factors and preparation for their samples to ensure that peptide levels are detectable and within the linear range of the standard curve.

12. Add 25µl of rehydrated primary antibody into each well except the blank wells (A1 and A2).

Note: A multi-channel pipette is NOT recommended to load the primary antibody.

13. Add 25µl of rehydrated biotinylated peptide into each well except the blank wells (A1 and A2).

Note: A multi-channel pipette is NOT recommended to load the biotinylated peptide.

14. Seal the microplate with an acetate plate sealer (APS). Incubate the microplate for 2 hours at room temperature (20-23°C).

Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

15. Centrifuge the SA-HRP vial (3,000-5,000 rpm for 5 seconds) to mix. Pipette 12µl of SA-HRP into 12ml of 1x assay buffer and vortex the solution thoroughly.
16. Remove the APS from the microplate and discard the contents of the wells. Wash each well with 350µl of 1x assay buffer, discard the buffer, invert the microplate, and blot the plate dry. Repeat 3 more times.
17. Add 100µl of SA-HRP solution into each well.
18. Reseal the microplate with an APS. Incubate for 1 hour at room temperature (20-23°C).

Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

19. Remove the APS from the microplate and discard the contents of the wells. Wash each well with 350µl of 1x assay buffer, discard the buffer, invert the microplate, and blot the plate dry. Repeat 3 more times.
20. Add 100µl of the TMB substrate solution into each well.

Note: TMB is light-sensitive. After the addition of the TMB substrate solution, it is strongly recommended to cover the microplate to protect it from light.

21. Reseal the microplate with an APS. Incubate for 1 hour at room temperature (20-23°C).

Note: Orbital shaking at 300-400 rpm is recommended for the duration of the incubation.

22. Remove the APS from the microplate. Do NOT wash the microplate or discard the contents of the wells.
23. Add 100µl of 2N HCl into each well to stop the reaction. The color in the wells should change from blue to yellow. Gently tap the plate to ensure thorough mixing.

Note: Proceed to the next step within 20 minutes.

24. Load the microplate onto a microtiter plate reader and measure absorbance O.D. at 450nm.

Calculation

1. Label the X-axis (log scale) with the concentration of standards #4 through stock (0.01 to 1000 ng/ml).
2. Label the Y-axis (linear scale) as absorbance (O.D.) at 450nm.
3. Average all duplicate readings (standards, positive control, samples) and substract the average blank O.D. reading.
4. Plot the O.D. for each standard peptide concentration directly above its X-axis coordinate.
5. Draw the best fi t curve through these data points. It should show a direct relationship between peptide concentration and absorbance. As the standard peptide concentration increases, the yellow color decreases, thereby reducing absorbance (O.D.).

Note: We strongly recommend using curve-fitting software capable of 4 parameter logistics or log-logit functionality.

6. To determine the peptide concentration in any unknown samples, first locate its absorbance (O.D.) on the Y-axis. Draw a horizontal line across the graph from that absorbance to the intersection with the standard curve. The X-axis coordinate at this intersection point will correspond to the peptide concentration (ng/ml) in the assayed sample.

Note: Multiply the measured peptide concentration by any dilution factor(s) used while preparing the original sample.

7. Refer to the QC Data Sheet for acceptable values of the positive controls.

Typical Standard Curve

Precision

Intra-assay variation: <10%

Inter-assay variation: <15%

Sensitivity

1.29 ng/ml

Specificity

Irisin, Recombinant (Human, Rat, Mouse, Canine): 100

Irisin (42-112) (Human, Rat, Mouse, Canine): 100

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

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

Irisin (42-95) (Human, Rat, Mouse, Canine): 0

FNDC4 (47-101) (Human) / FNDC4 (43-97) (Mouse): 0

FNDC4 (103-158) (Human) / FNDC4 (99-154) (Mouse): 0

FNDC4 / FRCP1 (190-234) (Human): 0

Linearity

1.29 - 27.5 ng/ml

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