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FNDC5 (Human) ELISA Kit

08/20

(Catalog # E4860-100; 96 assays, Storage at 4°C)

I. Introduction:

Fibronectin Type III Domain Containing 5 (Irisin) is a newly discovered hormone, secreted into circulation by muscle, which acts on white adipocytes and promotes WAT "browning". Irisin can reduce body weight and cause induction of brown adipocyte in vivo, including a capacity for thermogenic energy expenditure mediated by uncoupling protein 1 (UCP-1). Irisin mature protein is comprised of 110 amino acid residues and two potential glycosylation sites. BioVision's FNDC5 (Human) ELISA Kit is based on the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to human FNDC5. Standards or samples are added to the micro ELISA plate wells that bind to the specific antibody. Then a biotinylated detection antibody specific for human FNDC5 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The wells are washed, a TMB substrate solution is added to the wells and blue color develops in proportion to the amount of FNDC5 bound. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of human FNDC5. The concentration of human FNDC5 in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

in vitro quantitative determination of human FNDC5 concentrations in serum, plasma and other biological fluids.
Sensitivity: 0.10 ng/mL
Detection Range: 0.16-10 ng/mL
Specificity: No significant crossreactivity or interference between human FNDC5 and analogues was observed.
Precision: Coefficient of variation is < 10%.

III. Sample Type:

Serum, plasma and other biological fluids

IV. Kit Contents:

Components	E4860-100	Part Number	Storage
Micro ELISA Plate	8 wells x12 strips	E4860-100-1	-20°C
Reference Standard	2 vials	E4860-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4860-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4860-100-4	-20°C (protect from light)
Reference Standard & Sample Diluent	20 ml	E4860-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4860-100-6	4°C
HRP Conjugate Diluent	14 ml	E4860-100-7	4°C
Wash Buffer (25X)	30 ml	E4860-100-8	4°C
Substrate Reagent	10 ml	E4860-100-9	4°C (protect from light)
Stop Solution	10 ml	E4860-100-10	4°C
Plate Sealer	4	E4860-100-11	4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

An unopened kit can be stored at 2-8°C for 1 month. If the kit is not used within 1 month, store the items separately according to the above mentioned conditions once the kit is received.

VII. Reagent and Sample Preparation:

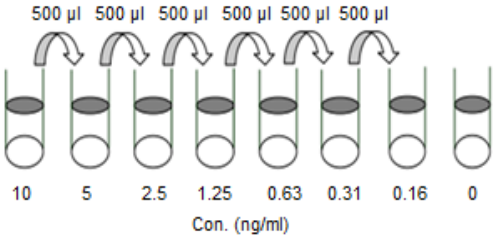
Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (25X):** Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. (Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved)

- **Biotinylated Detection Antibody working solution:** Calculate the required amount (100 µL/well). Centrifuge the stock tube before use; dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.

- **HRP Conjugate working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

- **Standard:** Centrifuge the standard at 10,000xg for 1 min. Add 1.0 ml of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 10 ng/ml. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10, 2.5, 1.25, 0.63, 0.32, 0.16, 0 ng/mL. Prepare 7 tubes; add 500 µl of Standard and Sample Diluent to each tube. Pipette 500 µl of the 10 ng/ml stock solution to the first tube and mix up to produce a 5 ng/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.



VIII. Sample Preparation:

- **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 2-8°C before centrifugation for 15 min at 1000xg at 2-8°C. Collect the supernatant. Blood collection tubes should be disposable and be endotoxin free.

- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 2-8℃ within 30 min of collection. Collect the supernatant. Hemolysed samples are not suitable for ELISA assay!

- **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000xg. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500xg at 2-8℃. Remove the cell fragments; collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

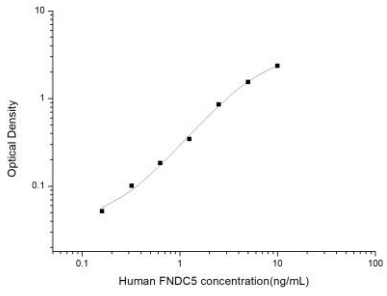
- **Tissue homogenates:** It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.

- **Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µL for each well). Add the samples to the other wells (100 µL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C.
   Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible
2. Remove the liquid out of each well, do not wash. Immediately add 100 µl of **Biotinylated Detection Antibody working solution** to each well. Cover with the Plate sealer. Incubate for 1 hr at 37°C.
3. Aspirate or decant the solution from each well, add 350 µl of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. **Note:** the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.



X. Calculation:

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Concentration(ng/mL)	10	5	2.5	1.25	0.63	0.32	0.16	0
OD	2.449	1.633	0.946	0.435	0.274	0.191	0.142	0.09
Corrected OD	2.359	1.543	0.856	0.345	0.184	0.101	0.052	-

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.

XI. Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and high level Human FNDC5 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and high level Human FNDC5 were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.51	0.87	4.57	0.51	0.86	4.33
Standard deviation	0.03	0.05	0.14	0.03	0.05	0.23
C V (%)	5.88	5.75	3.06	5.88	5.81	5.31

XII. Recovery:

The recovery of Human FNDC5 spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	**Range (%)**	**Average Recovery (%)**
Serum (n=5)	87-101	93
EDTA plasma (n=5)	90-102	96
Cell culture media (n=5)	87-98	93

XIII. Linearity:

Samples were spiked with high concentrations of Human FNDC5 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	**EDTA plasma (n=5)**	**Cell culture media (n=5)**
1:2	Range (%)	93-109	85-96	93-109
	Average (%)	101	91	100
1:4	Range (%)	87-100	83-96	85-101
	Average (%)	93	89	92
1:8	Range (%)	88-101	80-94	84-98
	Average (%)	94	86	89
1:16	Range (%)	91-107	86-96	88-99
	Average (%)	98	91	93

XIV. RELATED PRODUCTS:

- Irisin, Active, human recombinant (7852)
- Irisin Competitive ELISA Kit (K4761)
- FNDC5, human/mouse recombinant (7574)
- FNDC4, human/mouse recombinant (7573)

FOR RESEARCH USE ONLY! Not to be used on humans.