FOR RESEARCH USE ONLY!

Irisin Competitive ELISA Kit

(Catalog # K4761-100, 100 assays; Store at 4^{0}C) *rev 12/16*

I. Introduction:

BioVision's Irisin (human) Competitive ELISA kit is to be used for the in vitro quantitative determination of human irisin in cell culture supernatants, serum and plasma. It should also work for the in vitro quantitative determination of irisin in mouse, rat and monkey biological samples. A polyclonal antibody recognizing native irisin reacts with a series of predetermined recombinant irisin standard proteins or samples under competition in the irisin-coated plate. Their relative reactivity is plotted with that of the standard proteins. Detection limit: 1 ng/ml. Note: The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard. Assay Range: 0.001 μg/ml – 5 μg/ml. Irisin levels range in human plasma and serum from 0.2 to > 2 μg /ml.

II. Specificity:

This ELISA is specific for the measurement of natural and recombinant irisin in human samples. It should also work in mouse, rat and monkey biological samples. It does not cross-react with FNDC4, human adiponectin, human Nampt, human RBP4, human clusterin, human leptin, human vaspin, human GPX3, human resistin, human ACE2, human lipocalin-2, human ANGPTL3, human ANGPTL6, human DNER, human DLK1, human calreticulin, human IL-33, mouse Nampt, mouse clusterin, mouse vaspin, mouse resistin.

III. Sample Type:

- Serum & plasma
- Cell culture supernatants

IV. Kit Contents:

Components	K4761-100	Part Number
Plate coated with Irisin Recombinant Protein	6 x 16-well strips	K4761-100-1
Wash Buffer (10X)	2 x 30 ml	K4761-100-2
ELISA Buffer (10X)	2 x 30 ml	K4761-100-3
Detection Antibody	30 µl	K4761-100-4
HRP 100X (HRP Conjugated anti-rabbit IgG)	150 µl	K4761-100-5
Irisin Standard (lyophilized)	5 µg	K4761-100-6
TMB Substrate Solution	12 ml	K4761-100-7
Stop Solution	12 ml	K4761-100-8
plate sealers	2	K4761-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader at 450 nm, with the correction wavelength set at 540 nm
- Deionized water.
- Microtubes or equivalent for preparing dilutions.
- Disposable plastic containers for preparing working buffers

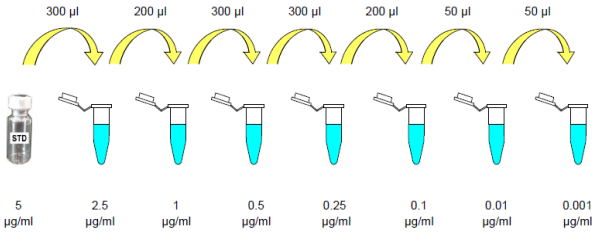
VI. Storage and Handling:

Reagent must be stored at 2-8°C when not in use. Plate and reagents should be at room temperature before use. Do not expose reagents to temperatures greater than 25°C.

VII. Reagent Preparation & Storage:

Note: Prepare just the appropriate amount of the buffers necessary for the assay.

- **Wash Buffer 10X:** Dilute with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain 1X Wash Buffer.
- **ESLIA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.
- **Detection Antibody (DET)** has to be diluted to 1:625 in ELISA Buffer 1X (16 µl DET + 10 ml ELISA Buffer 1X). NOTE: The diluted Detection Antibody is not stable and cannot be stored.
- **HRP 100X (HRP Conjugated anti-rabbit IgG)** has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100). NOTE: The diluted HRP is used within one hour of preparation.
- **Irisin Standard (STD)** has to be reconstituted with 1 ml of deionized water. This reconstitution produces a stock solution of 5 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 min. Mix well prior to making dilutions. **Note:** The reconstituted standard is aliquoted and stored at -20°C. Dilute the standard protein concentrate (STD) (5 µg/ml) in 1X ELISA Buffer. A seven-point standard curve in 1X ELISA Buffer is recommended. Suggested standard points are: 5, 2.5, 1, 0.5, 0.25, 0.1, 0.01 and 0.001 µg/ml.
- **Sample collection, storage and dilution**
  a) Serum: Use a serum separator tube. Let samples clot at room temperature for 30 min. before centrifugation for 20 min. at 1,000 x g. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated



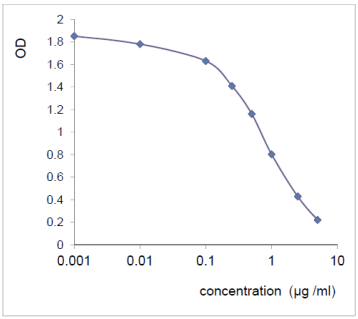
To obtain	Add	Into
5 µg/ml	-	-
2.5 µg/ml	300 µl of Irisin (5 µg/ml)	300 µl of ELISA Buffer 1X
1 µg/ml	200 µl of Irisin (2.5 µg/ml)	300 µl of ELISA Buffer 1X
0.5 µg/ml	300 µl of Irisin (1 µg/ml)	300 µl of ELISA Buffer 1X
0.25 µg/ml	300 µl of Irisin (0.5 µg/ml)	300 µl of ELISA Buffer 1X
0.1 µg/ml	200 µl of Irisin (0.25 µg/ml)	300 µl of ELISA Buffer 1X
0.01 µg/ml	50 µl of Irisin (0.1 µg/ml)	450 µl of ELISA Buffer 1X
0.001 µg/ml	50 µl of Irisin (0.01 µg/ml)	450 µl of ELISA Buffer 1X

freeze/thaw cycles.

b) Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min. at 1000 x g within 30 min. of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
Serum, Plasma or **Cell Culture Supernatant** have to be diluted in 1X ELISA Buffer. Samples containing visible precipitates must be clarified before use.
NOTE: As a starting point, 1/4 dilution of serum or plasma is recommended! If samples fall the outside range of assay, a lower or higher dilution may be required!

IX. Assay Protocol:

1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C. **NOTE:** Remaining 16-well strips coated with irisin protein when opened can be stored at 4°C for up to 1 month.
2. Add 50 µl of the different standards into the appropriate wells in duplicate! At the same time, add 50 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells (see reagent preparation).
3. Add 50 µl to each well of the Detection Antibody and tap gently on the side of the plate to mix.
4. Cover the plate with plate sealer and incubate for 1 hr at 37°C.
5. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multi-channel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
6. Add 100 µl to each well of the diluted HRP Conjugated anti-rabbit IgG (HRP) (see reagent preparation).
7. Cover the plate with plate sealer and incubate for 1 hr at 37°C.
8. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multi-channel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
9. Add 100 µl to each well of TMB substrate solution.
10. Allow the color reaction to develop at room temperature in the dark for 20 min.
11. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added. **Caution**: Corrosive solution.
12. Measure the OD at 450 nm in an ELISA reader within 30 min.
13. **Calculations:** Average the duplicate readings for each standard, control and sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding irisin concentration (µg/ml) on the horizontal (X) axis. Calculate the irisin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a 4-parameter logistic equation. If the test sample were diluted, multiply the interpolated value by the dilution factor to calculate the concentration of human irisin in the samples.



Standard Irisin (µg/ml)	Optical Density (mean)
5	0.219
2.5	0.428
1	0.803
0.5	1.161
0.25	1.409
0.1	1.631
0.01	1.781
0.001	1.882

Figure 1: Standard Curve:

Samples	Means (µg/ml)	SD	CV (%)	n
1	0.678	0.033	4.863	8
2	0.878	0.072	8.193	8
3	1.370	0.105	7.635	8
4	0.437	0.035	7.980	8
5	0.440	0.027	6.036	8
6	1.539	0.104	6.748	8

Table 1: **Intra-assay precision**: Six human samples of known concentration of irisin were assayed in replicates 8 times to test precision within an assay.

Samples	Means (µg/ml)	SD	CV (%)	n
1	0.532	0.051	9.673	5
2	1.145	0.092	8.027	5
3	0.725	0.060	8.254	5
4	0.731	0.071	9.656	5
5	0.696	0.068	9.719	5

Table 2: **Inter-assay precision:** 5 human samples of known concentration of irisin were assayed in 5 separate assays to test precision between assays.

Samples	Average recovery (%)	Range (%)
1	109.897	85-115
2	119.475	95-125
3	99.577	85-110

Table 3: When human samples (serum) are spiked with known concentrations of irisin, the recovery averages 109% (range from 85% to 120%).

Samples	Sample Dilution	Expected (µg/ml)	Observed (µg/ml)	% of Expected
1	1 : 2	0.229	0.229	100
	1 : 4	0.115	0.121	105.677
2	1 : 2	0.346	0.346	100
	1 : 4	0.173	0.175	101.156
3	1 : 2	0.232	0.232	100
	1 : 4	0.116	0.107	92.241
4	1 : 2	0.282	0.282	100
	1 : 4	0.141	0.145	102.837

Table 4: Different human serum samples containing irisin were diluted several fold (1/2 to 1/4) and the measured recoveries ranged from 90% to 107%.

X. RELATED PRODUCTS:

FTO (mouse intracellular) ELISA Kit (K4922)

FTO (human intracellular) ELISA Kit (K4921)

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