

## HIGH SENSITIVITY IRISIN (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF  
HUMAN THE IRISIN CONCENTRATIONS IN  
PLASMA AND SERUM



ALWAYS REFER TO LOT SPECIFIC  
PROTOCOL PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN  
DIAGNOSTIC PROCEDURES.

### PRODUCT INFORMATION:

**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HIGH SENSITIVITY IRISIN (HUMAN) ELISA KIT
Catalog No.	SK00170-08
Formulation	96 T
Lot No.	20114965
Standard range	0.2 ~ 12.8 ng/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, Plasma
Specificity	Human
Calibration	Glycosylated Irisin (Human) His Tag Rec. (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8 °C for 8 months. See page 3 for detail
This kit contains sufficient materials to run 35-40 samples duplicated provided that assay is run according to protocol.	

### Order Contact:

AVISCIERA BIOSCIENCE, INC.

2348 Walsh Ave., Suite C

Santa Clara, CA 95051

Tel: (408) 982 0300

Email: [Sales@AvisceraBioscience.com](mailto:Sales@AvisceraBioscience.com)

[Info@AvisceraBioscience.com](mailto:Info@AvisceraBioscience.com)

Website: [www.AvisceraBioscience.com](http://www.AvisceraBioscience.com)

[www.AvisceraBioscience.net](http://www.AvisceraBioscience.net)

**DESCRIPTION**

This High Sensitivity Irisin (Human) ELISA Kit contains the necessary components required for the quantitative measurement of human recombinant Irisin (HEK293) and/or natural the Irisin from serum and plasma in a sandwich ELISA format.

Due the amino acid sequence of human Irisin was identical to bovine, mouse, rat or other animals, please use animal free cell culture media for Irisin sample assay.

This immunoassay contains recombinant glycosylated human Irisin (HEK293) and monoclonal antibodies raised, selected and validated by this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural the Irisin samples.

**ASSAY OVERVIEW**

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Irisin. The capture antibody can bind to the human the irisin in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human the Irisin is added to the wells. After another washing of the plate, the streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human the Irisin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

**PROCEDURAL LIMITATIONS**

\_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix reagents with those from other lots or sources.

\_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

**COMPONENTS PROVIDED**

DESCRIPTION	CODE	QUANTITY
<b>Irisin Microplate</b> – 96 well microplate coated with monoclonal antibody specific for the human Irisin.	<b>170-08-01</b>	<b>1 plate</b>
<b>Irisin Standard</b> –51.2 ng of lyophilized recombinant human Irisin (Human cells).	<b>170-08-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.2 mL of 10-fold concentrate of lyophilized biotinylated monoclonal antibody against human Irisin.	<b>170-08-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of lyophilized recombinant human Irisin (human cells).	<b>170-08-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 45 mL of buffered solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>Antibody Diluent Solution</b> – 12 mL of buffered solution with preservative.	<b>DB11C</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> – 12 mL of buffered solution with preservative.	<b>DB08B</b>	<b>1 bottle</b>
<b>Wash Buffer</b> – 25 mL of 20-fold concentrated buffered surfactant with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> – 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.25M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

**STORAGE**

**Unopened Kit:** Store at 2 – 8 °C for up to 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored at -20 °C. Streptavidin-HRP Conjugate concentrate and TMB Substrate Solution can be stored only at 2 – 8 °C. Do not use kit past expiration date.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

**PRECAUTION**

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

**SAMPLE COLLECTION AND STORAGE**

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Cell Culture Samples** - The amino acid sequence of bovine Irisin is 100% identical to human Irisin; therefore, any culture media that contains fetal bovine serum or other animal serum **cannot** be used for Irisin assay. **Please use animal free culture media.**

**SAMPLE PREPARATION**

Serum and or plasma samples may require 4 ~ 8 fold dilution.

A suggested 4-fold dilution is 25 µl per well of sample + 75 µl per well of Dilution Buffer (DB10). A suggested 8-fold dilution is 12.5 µl per well of sample + 87.5 µl per well of Dilution Buffer.

**Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

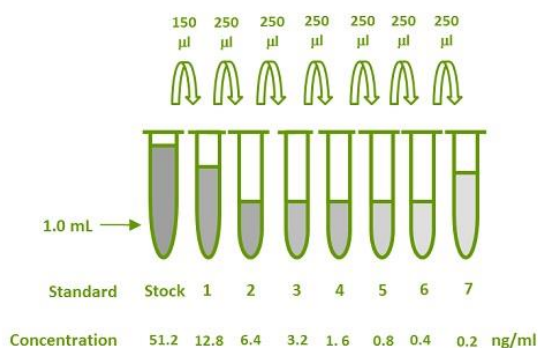
**REAGENT PREPARATION**

**Bring all reagents to room temperature before use.**

**Wash Buffer** – Dilute 25 mL of Wash Buffer Concentrate 20X into 475 mL distilled or deionized water to make 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved.

**Irisin Standard** – Reconstitute the human Irisin standard with 1 mL of **Dilution Buffer (DB10)** to this stock standard vial. The concentration of the reconstituted stock solution is 51.2 ng/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **12.8 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL). Store the stock solution at -70°C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1000µL	51.2 ng/mL
# 1	150 µL of stock	450 µL	12.8 ng/mL
# 2	250µL of 1	250µL	6.4 ng/mL
# 3	250µL of 2	250µL	3.2 ng/mL
# 4	250µL of 3	250µL	1.6 ng/mL
# 5	250µL of 4	250µL	0.8 ng/mL
# 6	250µL of 5	250µL	0.4 ng/mL
# 7	250µL of 6	250µL	0.2 ng/mL



**Positive Control** - Reconstitute the Positive Control with 1 mL of **Dilution Buffer (DB10)** to prepare working solution. Discard the positive control after use. It is for one time use only.

**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.2mL of **Antibody Diluent Solution (DB11C)** to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. For 96 wells test, Freshly Pipette 9.45 mL of **Antibody Diluent Solution (DB11C)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution and used in 10~20 minutes. *If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.*

**Streptavidin-HRP Conjugate** – For 96 wells test, Freshly Pipette 10.89 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**). **DO NOT FREEZE**. 1 x working solution should be used within 10-20 minutes. *If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 100-fold concentrated detection antibody at 2 ~ 8 °C for 12 months.*

## ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples

should be assayed in duplicate. **ELISA Protocol may need further optimization.**

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution, **samples**, or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker (250-300 rpm) at room temperature. Optional incubate for overnight at 2 ~ 8 °C without shaker.
4. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
5. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 90 minutes on microplate shaker (250-300rpm) at room temperature.
6. Repeat the aspiration and wash as in step 4.
7. Add 100 µL per well of **Streptavidin-HRP Conjugate working solution**. Cover with plate sealer and incubate for 45 minutes on microplate shaker at room temperature. **Protect from light**.
8. Repeat the aspiration and wash as in step 4.
9. Add 100 µL per well of **Substrate Solution**. Incubate for 10 min on microplate shaker at room temperature. **Protect from light**.
10. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read plate using a microplate reader set to 450 nm within 3 minutes.

## CALCULATION OF RESULTS

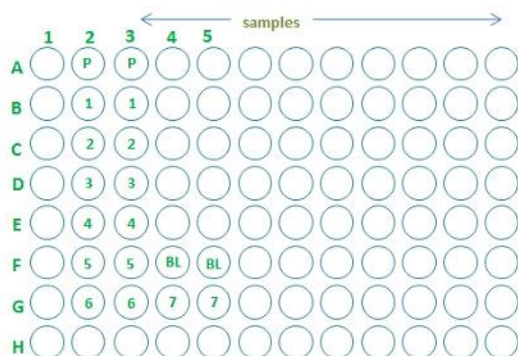
Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**SPECIFICITY**

PROTEIN	CROSS-REACTIVITY
Irisin (Human) (HEK293)	100%
Human PEDF	0
Human Myonectin	0
Human SPARC	0
Human FNDC4	0

The recombinant Irisin (Human) his tag derived from E. Coli was showed the cross-reactivity with this kit.



**TYPICAL STANDARD CURVE**

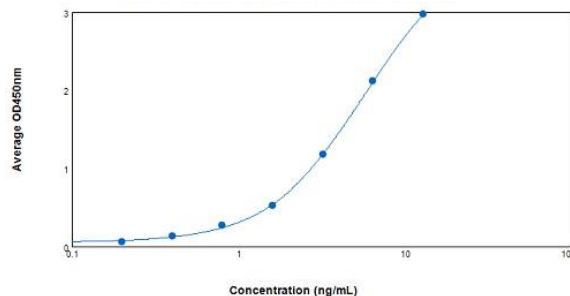
This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450nm (CORRECTED)
Blank	0 (0.097)
0.2	0.060
0.4	0.123
0.8	0.256
1.6	0.531
3.2	1.140
6.4	2.101
12.8	2.959

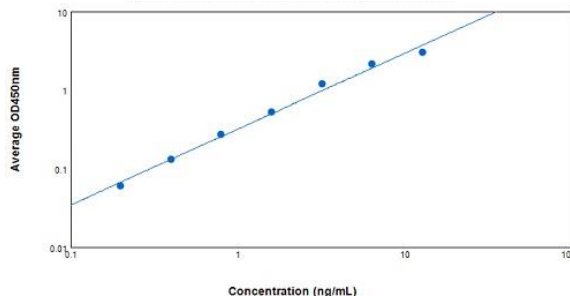
Lot No.: 20114965

Positive control: 1.8 ~ 5 ng/mL (log-log)

Standard curve (0.2~12.8 ng/mL) fit by 4-parameter:



Standard curve (0.2~12.8 ng/mL) fit by log-log:



**SUMMARY OF ASSAY PROCEDURE**

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT. Or incubate for overnight at 2~ 8 °C without shaker.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 90 minutes on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate 45 minutes on microplate shaker at RT. <b>Protect from light.</b>
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 10 min on microplate shaker at RT. <b>Protect from light.</b>
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 3 minutes.

The most cited and or new Biomarker ELISA KITS were manufactured by Aviscera Bioscience for Research Use Only.

Biomarker Name	Catalog No.
FAM19A1 Human ELISA Kit	SK00419-01
BMP8B Total Human ELISA Kit	SK00017-01
CTRP15/Monectin Human ELISA Kit	SK00393-15
CTRP7 Mouse ELISA Kit	SK00396-03
Heart Cadherin/CDH13 Human ELISA Kit	SK00839-06
OBP2A/Lipocalin 13 Human ELISA Kit	SK00648-08
OBP2A/Lipocalin 13 Mouse ELISA Kit	SK00648-01
Myonectin/CTRP15 Human ELISA Kit	SK00393-15
ENTPD3/CD39L3 Human ELISA Kit	SK00837-06
ENTPD5 Human ELISA Kit	SK00829-01
NT5E/CD73 Human ELISA Kit	SK00842-06
Soluble CD163 Human ELISA Kit	SK00694-01
High Sensitivity Human ESM-1/Endocan ELISA Kit	SK00318-08
High Sensitivity sTREM-2 Human ELISA Kit	SK00218-12A
sTREM-2 Mouse Rat ELISA Kit	SK00218-30
High Sensitivity sCSF1R Human ELISA Kit	SK00144-08
sCSF1R Mouse ELISA Kit	SK00144-03
High Sensitivity sACE2 Human ELISA Kit	SK00707-08
Sortilin Human ELISA Kit	SK00472-01
Sortilin Mouse Rat ELISA Kit	SK00472-03
Fetuin A Rat ELISA Kit (Serum, Plasma)	SK00173-02
Fetuin A Mouse ELISA Kit (Serum, Plasma)	SK00173-04
Fetuin A Human ELISA Kit	SK00173-20
FGF23 CT Peptide Human ELISA Kit	SK00147-06
Vasostatin-2 Human ELISA Kit	SK00084-02
Tetranectin Human ELISA Kit	SK00502-09
High Sensitivity BDNF Human Rat ELISA Kit	SK00752-01
Mouse Rat BDNF ELISA Kit	SK00752-03
High Sensitivity Irisin Human ELISA Kit	SK00170-06
FNDC4 Human ELISA Kit	SK00386-06
FSTL1 Human ELISA Kit	SK00372-06
Mouse Rat Autotaxin/ENPP2 ELISA Kit	SK00526-01
FGF23 C Terminal Peptide ELISA Kit	SK00147-16
FGF21 Mouse ELISA Kit	SK00145-03
Mouse ECP ELISA Kit	SK00128-03

High Sensitivity ECP Human ELISA Kit	SK00128-09
Soluble CD163 Human ELISA Kit	SK00694-01
Soluble CD320 Human ELISA Kit	SK00209-08
Soluble CD146 Human ELISA Kit	SK00628-01
Gas6 Rat ELISA Kit	SK00098-07
Hs-CRP Human ELISA Kit	SK00080-02
High Sensitivity BD5 Human ELISA Kit	SK00854-06
High Sensitivity BD4 Human ELISA Kit	SK00853-06
High Sensitivity BD1 Human ELISA Kit	SK00858-06
BD2 Human ELISA Kit	SK00856-06
BD3 Human ELISA Kit	SK00857-06

*FAM19A1 as a neuropeptide enables to control of food intake. BMP8B is a new adipokines secreted from adipocytes. Reliable new adipokines, myokines, Neurokines, Soluble Receptor and Secreted Proteins Immunoassay kits and its monoclonal antibodies are available in <https://www.aviscerabioscience.net>*