IRISIN (HUMAN) ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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	IRISIN (HUMAN) ELISA
Catalog No.	SK00170-09
Formulation	96 T
Lot No.	
Standard range	0.8-51.2 ng/mL
Sensitivity	100 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	Recombinant Irisin (Human) (human cells derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C for 1 month. See page 3 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Irisin (Human) ELISA Kit contains the necessary components required for the quantitative measurement of human recombinant Irisin (human cells derived) 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 (human cells derived) 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
Irisin Microplate – 96 well microplate coated with monoclonal antibody specific for the human Irisin.	**170-09-01**	**1 plate**
Irisin Standard –refer to lot of lyophilized recombinant human Irisin (Human cells).	**170-09-02**	**1 vial**
Detection Antibody Concentrate – refer to lot of 10-fold concentrate of lyophilized biotinylated antibody against human Irisin.	**170-09-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human Irisin (human cells).	**170-09-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered solution with preservative.	**DB108A**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB68C**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 1 month. For longer storage for up to 10 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 at least 8 ~32-fold dilution. A suggested 32-fold dilution is 10 µl sample + 310 µl **Dilution Buffer (DB09)**. 16-fold dilution is 20 µl sample + 300 µl **Dilution Buffer (DB09).** 8-fold dilution is 30 µl sample + 210 µl **Dilution Buffer (DB09)**.

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 50 mL of Wash Buffer Concentrate into 450 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.

Dilution Buffer (DB09) - **Dilution Buffer is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.**

Irisin Standard – Reconstitute the human Irisin standard with refer to lot of **Dilution Buffer (DB09)** 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 **51.2 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot	51.2 ng/mL
# 1	250 µL of stock	250 µL	25.6 ng/mL
# 2	250µL of 1	250µL	12.8 ng/mL
# 3	250µL of 2	250µL	6.4 ng/mL
# 4	250µL of 3	250µL	3.2 ng/mL
# 5	250µL of 4	250µL	1.6 ng/mL
# 6	250µL of 5	250µL	0.8 ng/mL

Positive Control - Reconstitute the Positive Control with refer to lot of **Dilution Buffer (DB09)** to prepare working solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody Diluent Solution (DB108A)** to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 9.45 mL of **Antibody Diluent Solution (DB108A)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB68C)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

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 (400-450 rpm) at room temperature.
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 2 hours on microplate shaker (400-450rpm) 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 60 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 refer to lot 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 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) (human cells derived)	100%
Human PEDF	0
Human Myonectin	0
Human SPARC	0
Human IL-13	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 (refer to lot)
0.4 (optional)	0.040
0.8	0.070
1.6	0.128
3.2	0.257
6.4	0.456
12.8	0.905
25.6	1.801
51.2	3.099

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.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 2 hours 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 60 minutes on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate refer to lot on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 3 minutes.