IRISIN (HUMAN, MOUSE, RAT) ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEM OF EACH KIT BEFORE USING THIS PRODUCT.

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

PURCHASE INFORMATION:

ELISA NAME	IRISIN (Human, Mouse, Rat) ELISA KIT
Catalog No.	SK00170-01
Lot No.	
Formulation	96 T
Standard range	0.066 - 1024 ng/mL
Dynamic range	1.638 – 1024 ng/mL
Sensitivity	~1 ng/mL
Sample Volume	50 µL
Dilution Factor	2 or 4 for serum or plasma samples (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma
Specificity	Human, Mouse, Rat Irisin
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C for 1 month. See page 3 for detail

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INTRODUCTION

Irisin (Human, Mouse, Rat) ELISA employs the quantitatively competitive enzyme immunoassay technique in which Irisin present in samples compete with a fixed amount of biotinylated Irisin for sites on purified rabbit IgG specific against Irisin. During the incubation period, the rabbit IgG specific for Irisin binds to the goat anti-rabbit IgG pre-coated onto the microplate. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when stop solution is added. The intensity of the color measured is in inverse proportion to the amount of Irisin bound in the initial step. The sample values are then read off the standard curve.

Irisin (Human, Mouse, Rat) ELISA has been shown to accurately quantify the recombinant and natural Irisin. Results obtained using natural Irisin showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_Some vials contain small quantities of material, therefore centrifuge before use.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well microplate pre-coated with polyclonal anti rabbit IgG Fc.	**PRM01**	**1 plate**
Irisin Standard – 1024 ng/vial of recombinant Irisin in a buffered protein base with preservative; lyophilized.	**170-01-01**	**1 vial**
Irisin Biotin – 1.5 mL of 6-fold concentrated biotinylated Irisin with preservative; lyophilized.	**170-01-02**	**1 bottle**
Irisin Antibody – 1.5 mL of 6-fold concentrated polyclonal purified IgG against Irisin with preservative; lyophilized.	**170-01-03**	**1 bottle**
Positive Control – one vial of recombinant Irisin; lyophilized (optional).	**170-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative. Ready to use.	**DB11C**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative. Ready to use.	**DB08B**	**1 bottle**
Blue Solution- 200 µL of Blue Color Solution	**B01**	**1 vial**
Yellow Solution-200 µL of Yellow color solution	**Y01**	**1 vial**
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 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 1 month. For longer storage for up to 6 months, unopened Standard, Positive Control, Irisin Antibody and Irisin Biotin, Dilution Buffer and HRP Diluent Solution should be stored at -20 °C.
Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Irisin Antibody and Irisin Biotin SHOULD BE STORED at -20 °C or -70 °C for up to one month. Reconstituted Irisin Biotin CANNOT BE STORED at 2-8 °C. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8 °C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

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

For cell cultures supernates, must use ***animal serum free media***. Fetal bovine serum samples cross-reacts with this ELISA Kit.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 2-fold or 4-fold dilution. A suggested 2-fold dilution is 75 µL sample + 75 µL Dilution Buffer. A suggested 4-fold dilution is 40 µL sample + 120 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

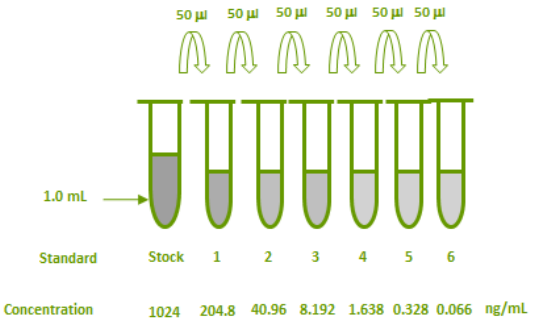
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Irisin Standard - Refer to vial label for reconstitution volume. Reconstitute the **Irisin standard** with 1.0 mL of Dilution Buffer (DB11C). This reconstitution produces a stock solution of 1024 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1024 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL) which was named as total binding.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0mL	1024 ng/ml
# 1	50µl of stock	200µl	204.8 ng/ml
# 2	50µl of 1	200µl	40.96 ng/ml
# 3	50µl of 2	200µl	8.192 ng/ml
# 4	50µl of 3	200µl	1.638 ng/ml
# 5	50µl of 4	200µl	0.328 ng/ml
# 6	50µl of 5	200µl	0.066 ng/ml
Total Binding	0	250µl	0 ng/mL



Positive Control - Reconstitute the **Positive Control** with 1.0 mL of **Dilution Buffer (DB11C)**. **Note:** Positive Control could be reused within 2 ~ 3 days if stored at -20 °C ~ -70 °C.

Irisin Antibody – Reconstitute the **Irisin Antibody** with **1.5 mL** of **Dilution Buffer (DB11C)** to produce a 6 -fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 5 mL of **Dilution Buffer (DB11C)** into a 15 mL centrifuge tube and transfer 1 mL of 6-fold concentrated stock solution to prepare 1x Antibody working solution. **Optional add 100 µL of Blue Solution into 6.0 mL of 1x Antibody Solution. It is 50 µL per well.**

Irisin Biotin - Reconstitute the **Irisin Biotin** with 1.5 mL of **Dilution Buffer (DB11C)** to produce a 6 -fold concentrated stock solution. Pipette 5 mL of **Dilution Buffer (DB11C)** into a 15 mL centrifuge tube and transfer 1 mL of 6-fold concentrated stock solution to prepare 1x Biotin working solution. **Optional add 100 µL of Yellow Solution into 6.0 mL of 1x Biotin Solution. It is 50 µL per well.**

Streptavidin-HRP Conjugate - Transfer 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB08B)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within 1-2 hours **(protect from light)**.

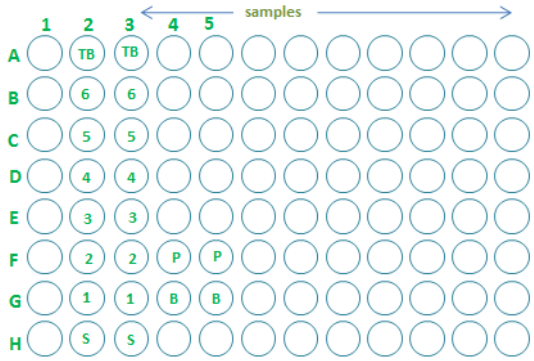
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave wells G4, G5 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set A2, A3 as total binding (TB). Add 50 µL per well of Dilution Buffer.
5. Add 50 µL per well of **standard solutions** from #6 to #S (reverse order of serial dilution) to the appropriate wells (B2, B3 to H2, H3). Add 50 µL per well of **Positive Control** into wells F4, F5. Add 50 µL per well of **samples** into appropriate wells.
6. Add 50 µL per well of **Blue Colored 1x Antibody Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (350-400rpm) at room temperature for 2 hours. **NOTE: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.**
7. Add 50 µL per well of **Yellow Colored 1x Biotin Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (350-400rpm) at room temperature for 2 hours. **NOTE: DO NOT ADD Biotin Solution to Blank wells.**
8. Aspirate wells and wash 4 times with 300 µL of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well, including blanks.

Incubate on microplate shaker (350-400rpm) for 60 minutes at room temperature. **Protect from light**.

10. Aspirate and wash as step 8.
11. Add 100 µL of **Substrate Solution** to each well. Incubate on microplate shaker (350-400rpm) for 3-7 minutes at room temperature. **Protect from light**.
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
12. Determine the optical density of each well within 2 minutes using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D. absorbances. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 1024 ng/mL may result in inaccurate, low Irisin levels. Such samples require further external predilution according to expected Irisin values with Dilution Buffer in order to precisely quantify the actual Irisin level. **Optimal dilutions should be determined by each laboratory for each application.**

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Irisin.

SENSITIVITY

~1 ng/mL

TYPICAL DATA

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

LAYOUT	STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
BLANK		0 (0.059)
STOCK STD	1024	0.116
STD1	204.8	0.288
STD2	40.96	0.840
STD3	8.192	1.803
STD4	1.638	2.143
STD5	0.328	2.423
STD6	0.066	2.649
TOTAL BINDING	0	2.820

SPECIFICITY

This assay recognizes both natural and recombinant Irisin. The factors listed below were prepared at 500 µg/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY (%)
Irisin (Human, Mouse, Rat)	100
Human Chemerin	0
Human FGF-21	0
Human Visfatin	0
Human Vaspin	0
Human FABP4	0
Human SPARC	0
Human Omentin 1	0
Human ANGPTL4	0

The fetal bovine neat or diluted serum samples were detectable in this ELISA kit, which indicates that fetal bovine serum samples cross-reacts with this ELISA Kit. For cell culture supernates, must use animal serum free media.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 50 µL of standard, samples, positive control to the well. Add 50 µL of Blue Colored 1x Antibody Solution to each well used, except blanks. Incubate 2 hours on the plate shaker at RT. **DO NOT ASPIRATE AND WASH PLATE. PROCEED TO NEXT STEP.**
⬇
Add 50 µL of Yellow Colored 1x Biotin Solution to each well used, except blanks. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL Streptavidin-HRP conjugate working solution to all wells, including blanks. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL Substrate Solution to each well. Incubate 3-7 min on plate shaker at RT. **Protect from light.**
⬇
Add 100 µL Stop Solution to each well. Read 450nm within 2 min.