

ab285295– Human Irisin ELISA Kit

For the quantitative measurement of Irisin.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285295>

Storage and Stability

On receipt entire kit should be stored at 4°C, protected from light. Upon opening, use kit within 6 months. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Irisin Recombinant Protein pre-coated Microplate	6 x 16-well strips	4°C
Wash Buffer (10X)	2 x 30 mL	4°C
ELISA Buffer (10X)	2 x 30 mL	4°C
Detection Antibody	30 µL	4°C
100X HRP Conjugate anti-rabbit IgG	150 µL	4°C
Irisin Standard (lyophilized)	5 µg	4°C
TMB Substrate Solution	12 mL	4°C
Stop Solution	12 mL	4°C
Plate sealers	2 units	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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

Reagent Preparation

- 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

ELISA 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: must be diluted to 1:625 in ELISA Buffer 1X (16 µl DET + 10 ml ELISA Buffer 1X).

Δ Note: The diluted Detection Antibody is not stable and cannot be stored

100X HRP Conjugate anti-rabbit IgG: has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).

Δ Note: The diluted HRP is used within one hour of preparation

Standard 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.

Dilution number	Irisin Standard	ELISA Buffer	Final concentration (µg/ml)
1	-	-	5
2	300 µL (5 µg/ml)	300 µL	2.5
3	200 µL (2.5 µg/ml)	300 µL	1
4	300 µL (1 µg/ml)	300 µL	0.5
5	300 µL (0.5 µg/ml)	300 µL	0.25
6	200 µL (0.25 µg/ml)	300 µL	0.1
7	50 µL (0.1 µg/ml)	450 µL	0.01
8	50 µL (0.01 µg/ml)	450 µL	0.001

Sample Preparation

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 freeze/thaw cycles.

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.

Δ Note: Serum, Plasma or Cell Culture Supernatant have to be diluted in 1X ELISA Buffer. Samples containing visible precipitates must be clarified before use.

Δ 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!

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.

Calculation:

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 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.

Technical Support

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