SOLUBLE FNDC5 (HUMAN, MOUSE, RAT) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF SOLUBLE FNDC5 CONCENTRATIONS IN SERUM AND PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	SOLUBLE FNDC5 (HUMAN, MOUSE, RAT) ELISA KIT
Catalog No.	SK00169-02
Lot No.	
Formulation	96 T
Standard Range	1.25 - 320 ng/mL
Sensitivity	0.1 ng/mL
Sample Volume	100 µL
Sample Type	Serum and Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human, Mouse and Rat Soluble FNDC5. Cross-reacts with Irisin.
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2°C - 8°C for

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: info@aviscerabioscience.com
sales@aviscerabiosicence.com
www.AvisceraBioscience.com

INTRODUCTION

Soluble FNDC5 immunoassay is a solid phase ELISA designed to measure soluble FNDC5 in human,

mouse and rat, serum and plasma. Other samples, such as cell culture supernates, need to be validated prior to use. Please note, for cell culture supernates, must use animal serum free culture media. This kit contains recombinant soluble FNDC5 and antibodies raised against this protein. It has been shown to accurately quantify recombinant soluble FNDC5. Results obtained with naturally occurring soluble FNDC5 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural soluble FNDC5.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for soluble FNDC5 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any soluble FNDC5 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for soluble FNDC5 is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin HRP is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of soluble FNDC5 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 the appropriate 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.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Soluble FNDC5 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against soluble FNDC5.	169-02-01	1 plate
Soluble FNDC5 Standard – 320 ng/vial of recombinant soluble FNDC5 in a buffered protein base with preservative; lyophilized.	169-02-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated antibody against soluble FNDC5 with preservative; lyophilized.	169-02-03	1 vial
Positive Control – one vial of recombinant soluble FNDC5; lyophilized.	169-02-04	1 vial
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	SAHRP	1 vial
Dilution Buffer - 45 mL of buffered protein based solution with preservative.	DB08B	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
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 1 month. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate and Dilution Buffer should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8 °C for up to 10 months. **DO NOT FREEZE TMB SUBSTRATE SOLUTION.**
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 month 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.

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, heparin, or citrate 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

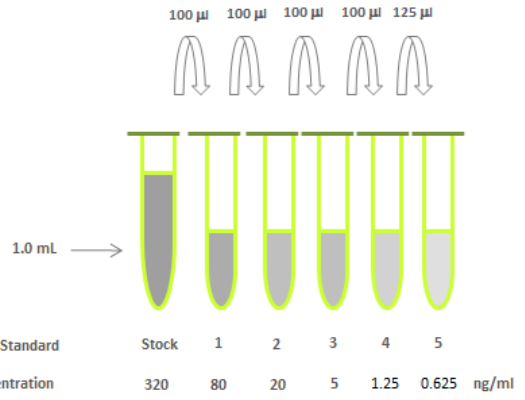
REAGENT PREPARATION

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.

Soluble FNDC5 Standard - Refer to vial label for reconstitution volume. Reconstitute the **Soluble FNDC5** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 320 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of the appropriate Dilution Buffer into tubes #1 to #4. For standard solution #5, it is a 2-fold dilution of standard solution #4, not 4-fold. Pipette 125 µL of the appropriate Dilution Buffer into tube #4. Use the stock solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The **320 ng/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	320 ng/ml
# 1	100 µl of stock	300 µl	80 ng/ml
# 2	100 µl of 1	300 µl	20 ng/ml
# 3	100 µl of 2	300 µl	5 ng/ml
# 4	100 µl of 3	300 µl	1.25 ng/ml



Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. Allow the positive control to sit for a minimum of 15 minutes with gentle agitation prior to assay. **Note:** Positive control could be reused within a few days if stored at -20 ~ -70 °C.

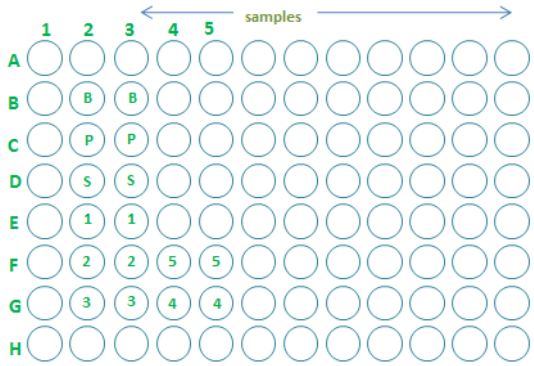
Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Vortex until completely dissolved. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 10.89 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days. **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. Add 100 µL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 µL of **Standard solutions** (reverse order of serial dilution; F4, F5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **TMB Substrate Solution** to each well. Incubate for 5-15 minutes on microplate shaker 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. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using the reader's software. It is recommended to use software capable of generating a log-log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the soluble FNDC5 concentrations versus the log of the absorbance and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human soluble FNDC5.

SENSITIVITY

The minimum detectable dose (MDD) of soluble FNDC5 was 0.1 ng/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	450NM READING (CORRECTED)
Blank	0 (0.190)
1.25	0.041
5	0.210
20	0.851
80	1.478
320	1.946

SPECIFICITY

This assay recognizes both natural and recombinant soluble FNDC5. The factors listed below were prepared at 32 µg/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY (%)
Soluble FNDC5 (H,M,R)	100
Irisin (H,M,R)	100
Human Adiponectin	0
Human Omentin 1	0
Human Vaspin	0
Human Leptin	0
Human FABP4	0
Human CTRP1	0
Human Periostin	0
Human SPARC	0
Human BDNF	0
Human FGF-21	0
Mouse FGF-21	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. Protect from light.

↓

Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate for 5-15 min on the plate shaker at RT. Protect from light.

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Add 100 µl Stop Solution to each well. Read signal at 450nm within 3 min.