HUMAN FOLLISTATIN-LIKE PROTEIN 3 (FSTL3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FOLLISTATIN-LIKE PROTEIN 3 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

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.

PURCHASE INFORMATION:

ELISA NAME	**HUMAN FSTL3 ELISA**
Catalog No.	SK00347-06
Lot No.	
Formulation	96 T
Standard range	78 - 10,000 pg/ml
Sensitivity	20 pg/ml
Sample require	100 µl
Dilution Factor	**2 - 4 (Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Specificity	Human FSTL3
Calibration	Human FSTL3 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Follistatin-Like Protein 3 ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Follistatin-like protein 3 in cell culture supernates, serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Follistatin-like protein 3 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Follistatin-like protein 3 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. An antibody specific for Follistatin-like protein 3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Follistatin-like protein 3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for Follistatin-like protein 3 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, a streptavidin-HRP conjugate 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 Follistatin-like protein 3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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.
_ 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
FSTL3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against Follistatin-like protein 3.	**347-06-01**	**1 plate**
FSTL3 Standard – 10,000 pg/vial of recombinant human Follistatin-like protein 3 in a buffered protein base with preservative; lyophilized.	**347-06-02**	**1 vial**
Detection Antibody– 1.05mL/vial of 10-fold concentrate of a biotinylated antibody against Follistatin-like protein 3 with preservative; lyophilized.	**347-06-03**	**1 vial**
Positive Control – one vial of recombinant Follistatin-like protein 3; lyophilized.	**347-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial of 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08**	**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 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate 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 8 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C or -70° 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.
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

Serum and plasma samples may require a 2 to 4-fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. A suggested 4-fold dilution is 60 µL sample + 180 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

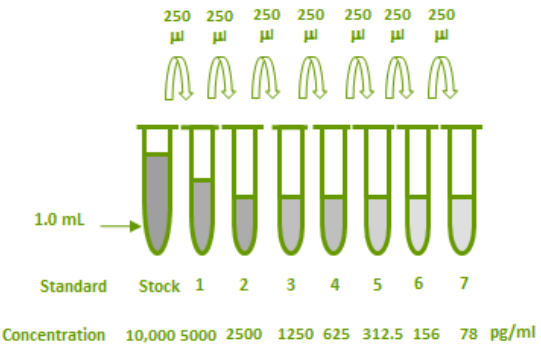
REAGENT PREPARATION

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.

FSTL3 Standard - Reconstitute the FSTL3 standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 10,000 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µl of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **10,000 pg/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/ml).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	10,000 pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	312.5 pg/ml
# 6	250µl of 5	250µl	156.25 pg/ml
# 7	250µl of 6	250µl	78.125 pg/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer. *Note: Positive Control could be reused within a few days if stored at -20° C or -70° C.*

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB08)** 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 micro-plate 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.
4. Add 100 µL of Standard dilution from #7 to #S (reverse order of serial dilution), sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
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 micro-plate 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 45 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes on micro-plate 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 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer 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 Follistatin-like protein 3 concentrations versus the log of the O.D. 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.

Calculation of samples with a concentration exceeding that of standard 10,000 pg/mL may result

in inaccurate, low human Follistatin-like protein 3 levels. Such samples require further external predilution according to expected human Follistatin-like protein 3 values with Dilution Buffer in order to precisely quantify the actual human Follistatin-like protein 3 level.

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human Follistatin-Like Protein 3	100%
Human Follistatin-Like Protein 1	0
Human Follistatin-Like Protein 4	0
Human Follistatin-Like Protein 5	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.064)
78.125	0.016
156.25	0.018
312.5	0.061
625	0.127
1250	0.168
2500	0.415
5000	1.032
10,000	1.843

- **Lot No.:**
- **Positive Control:**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 45 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 the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.