FSTL-3 ELISA

RUO

AL-152

INTENDED USE

The FSTL-3 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of FSTL-3 in human serum. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Follistatin-like 3 (FSTL-3), also known as Follistatin-related Gene (FLRG) and Follistatin-related protein (FSRP), is a 30-35 kDa secreted glycoprotein. FSTL-3 encodes a novel secreted glycoprotein that is highly homologous to Follistatin and binds activins and bone morphogenetic proteins, members of the TGF beta superfamily of growth/differentiation factors. FSTL-3 protein inhibits activin-induced and bone morphogenetic protein-2-induced transcriptional responses in a dose-dependent manner, and its mRNA is abundantly expressed in human placenta,[1] trophoblast,[2] heart,[3] uterus,[3,4] ovary,[5,6] testis[7] and adrenal gland.[6] Mature human FSTL-3 consists of an Activin and Myostatin binding N-terminal domain, two Follistatin-like domains, and two Kazal-like domains.[8-12] BMP-2, -6, and -7 do not compete for Activin A binding, and FSTL-3 binds only weakly to Activin B.[13,14] Unlike Follistatin, FSTL-3 does not contain a heparin-binding domain and does not interact with heparin sulfate proteoglycans.[15,16] FSTL-3 has been studied in regulation of energy balance and metabolism,[17] gestational diabetes[18,19] preeclampsia,[20] and promotor of tumor cell proliferation. [21,22]

PRINCIPLE OF THE TEST

The FSTL-3 ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to FSTL-3 antibody coated micro titer wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated FSTL-3 antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of FSTL-3 in the samples and calibrators.

MATERIALS SUPPLIED

CAL-152A FSTL-3 Calibrator A/Sample Diluent

One bottle, 11 mL, labeled FSTL-3 Cal A/Sample Diluent, containing 0 ng/mL FSTL-3 in a protein-based buffer with a non-mercury preservative. Store unopened at 2-8°C until the expiration date.

CAL-152B-CAL-152F FSTL-3 Calibrators B thru F (Lyophilized)

Five vials, labeled B-F containing concentrations of approximately 0.8 – 15.0 ng/mL FSTL-3 in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with **1.0 mL** deionized water. Solubilize for **10 minutes**, mix well and use after reconstitution. Discard after 5 days, if stored at 2 to 8°C. For longer storage aliquot and freeze at -20°C or colder.

CTR-152-I and CTR-152-II FSTL-3 Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high FSTL-3 in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact control ranges. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with **1.0 mL** deionized water. Solubilize for **10 minutes**, mix well and use after reconstitution. Discard after 5 days, if stored at 2 to 8°C. For longer storage aliquot and freeze at -20°C or colder.

PLT-152 FSTL-3 Antibody Coated Microtiter Plate Strips

One strip holder, containing 96 microtiter wells with FSTL-3 antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ASB-152 FSTL-3 Assay Buffer

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-152 FSTL-3 Biotin Conjugate Concentrate

One vial, 0.4 mL containing a solution of biotinylated detection antibody concentrate in a protein-based buffer with a non-mercury preservative. Dilute prior to use in FSTL-3 Conjugate diluent. Store at 2-8°C until expiration date.

CND-152 FSTL-3 Biotin Conjugate Diluent

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-152 FSTL-3 Streptavidin Enzyme Conjugate Ready to Use

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate absorbance reader capable of absorbance measurement at 450 nm, 405nm, and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Vortex mixer.
5. Repeater pipette.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.[23]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[24] as a preservative. Pro-Clean 400 and Sodium Azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples may be stored at 4°C if assayed within 24 hours; otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the FSTL-3 ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, Sodium Azide, Hypochlorous Acid and aromatic Chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **FSTL-3 Calibrators B-F and Controls I & II**: Tap and reconstitute FSTL-3 Calibrators B-F and Controls I & II each with 1 mL deionized water. Solubilize for 10 minutes, mix well and use after reconstitution.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **FSTL-3 Antibody-Biotin Conjugate Solution:** The FSTL-3 Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1-part conjugate to 50 parts of FSTL-3 Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220 µL of the Concentrate in to 11 mL of the Assay buffer.
4. **Microtiter Plate Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

Note: All samples should be diluted 1:5 (20 µL sample+ 80 µL* FSTL-3 Calibrator A/Sample Diluent*). Do not dilute the calibrators and controls.

1. Allow the calibrators to reach the room temperature (23 ± 2°C) & mix well by gentle vortex.
2. Label the microtiter strips to be used.
3. Pipette **25 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add **100 µL** of the FSTL-3 Assay Buffer to each well using a repeater pipette.
5. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
6. With 30-40 minutes remaining of incubation time, prepare the FSTL-3 Antibody-Biotin Conjugate Solution by diluting the FSTL-3 Biotin Conjugate Concentrate in FSTL-3 Conjugate Diluent as described under the Preparation of the Reagents section of this insert.
7. Aspirate and wash each strip **5 times** with Wash Solution (350 **µL**/per well) using an automatic microplate washer.
8. Add **100 µL** of the FSTL-3 Biotin Conjugate solution to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
10. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.

11. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
13. Aspirate and wash each strip **5 times** with the Wash Solution (350 **µL**/per well) using an automatic microplate washer.
14. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **10** ± 2 **minutes** at room temperature (23 ± 2°C).
    NOTE: Visually monitor the color development to optimize the incubation time.
16. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.
    NOTE: *While reading the absorbance of the microtiter wells, it is necessary to program the zero calibrator as a "Blank".*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the data on a log vs. log scale using a cubic regression curve-fit. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the FSTL-3 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the FSTL-3 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding FSTL-3 concentrations.
4. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A / Sample Diluent) and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. **Multiply the value by a dilution factor.**

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- FSTL-3 ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for FSTL-3 controls are printed on the Calibration card.
- A full calibration curve, low- and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance	Conc (ng/mL)
A1, A2	Calibrators A	0.014 (Blank)	0
B1, B2	B	0.029	0.4
C1, C2	C	0.076	1.1
D1, D2	D	0.549	3.7
E1, E2	E	1.571	7.4
F1, F2	F	3.858	14.0

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory*

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in ng/mL.

Analytical Sensitivity:

The lowest amount of FSTL-3 in a sample that can be detected with 12 assay runs performed over two days with samples run in duplicate per run(n=24) is 0.164 ng/mL.

Imprecision:

Reproducibility of the FSTL-3 ELISA assay was determined in a study using two serum pools. The study included a total of 12 assays, two replicates of each per assay (n=24). Representative data were calculated and are presented in the following table:

Sample	Mean conc.	Within run		Between run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	1.353	0.039	2.8%	0.011	0.8%	0.040	3.0%
Control II	3.668	0.102	2.8%	0.057	1.6%	0.117	3.2%
Sample 1	3.125	0.058	1.9%	0.032	1.0%	0.067	2.1%
Sample 2	2.894	0.100	3.5%	0.055	1.9%	0.114	3.9%

Linearity:

Based on dilutions of the four serum samples containing various FSTL-3 levels diluted with Calibrator A/sample diluent the % recovery on individual samples is represented in the following:

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	Neat	8.197	Neat	NA
	1:2	4.099	4.193	102%
	1:4	2.049	2.160	105%
	1:8	1.025	0.961	94%
2	Neat	6.451	Neat	NA
	1:2	3.226	3.299	102%
	1:4	1.613	1.702	106%
	1:8	0.806	0.900	112%
3	Neat	8.246	Neat	NA
	1:2	4.123	4.441	108%
	1:4	2.062	2.156	105%
	1:8	1.031	1.120	109%

Recovery:

Known amounts of FSTL-3 were added to two serum samples containing different levels of endogenous FSTL-3. The concentration of FSTL-3 was determined before and after the addition of exogenous FSTL-3 and the percent recovery was calculated.

Sample	Endogenous Conc.(ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1.090	6.545 4.727 3.272	6.888 5.273 4.091	105% 112% 125%
2	0.900	6.450 4.600 3.120	6.842 5.333 3.770	106% 116% 121%
3	1.745	2.462 2.223 2.032	2.464 2.292 1.993	100% 103% 98%
3	1.745	2.452 2.209 2.016	2.449 2.218 2.058	100% 100% 102%

Cross reactivity and specificity:

The monoclonal antibody pair used in the assay detects human, Caprine, Bovine, Equine and Canine FSTL-3. Closely related analytes when tested at the concentration shown in table below did not show any detectable cross-reactivity.

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	FS-288	100	ND
2	FS-315	100	ND
3	Activin A	100	ND
4	Activin B	100	ND

Interference:

When potential interferents (hemoglobin, triglycerides, bilirubin and Biotin) were added at least at two times their physiological concentration to control sample, FSTL-3 concentration was within ± 10% of the control as represented in the following table.

Interferents	Analyte Conc.	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35 mg/mL	12.78	12.74	-0.3
		16.96	17.88	5.4
Triglycerides	5.00 mg/mL	12.78	12.85	0.5
		16.96	17.80	5.0
Bilirubin	0.60 mg/mL	13.90	12.98	-6.6
		19.05	18.87	-0.9
Biotin	600 ng/mL	2.18	2.11	-3.1
		2.01	1.88	-6.3

Expected Value:

The expected concentration ranges (95% CI) for FSTL-3 in males and females were calculated and listed in the table below.

Population	Age Range (Yrs.)	No. of Specimens	Median FSTL-3 Conc. (ng/mL)	FSTL-3 Range (ng/mL)
Male	20-40	56	16.8	7.3 - 69.8
Male	41-54	92	22.4	8.5 – 75.9
Female	21-40	36	17.9	8.6 – 75.7
Female	41-55	50	17.8	7.9 – 75.0

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