# AnshLite™ Follistatin CLIA RUO

## AL-217

### INTENDED USE

The AnshLite™ Follistatin enzyme linked chemiluminescent assay (CLIA) kit provides materials for the quantitative measurement of follistatin in human serum and other biological fluids. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

### SUMMARY AND EXPLANATION

AnshLite™ Follistatin CLIA (FST) is a glycosylated single-chain protein that is expressed in a wide variety of tissues.[1] Activin stimulates pituitary FSH secretion whereas Inhibin and Follistatin are inhibitory.[2] Follistatin has been shown to be a potent activin-binding protein which acts by neutralizing the actions of the Activins.[3] The activin-follistatin binding complex is generally considered to be composed of one activin and two follistatin molecules, and the affinity of binding between follistatin and activin is similar to that of activin for its receptor. Several isoforms of follistatin of molecular weight 31 – 39 kDa have been identified. Molecular analysis of the isoforms indicate that follistatin is encoded by a single gene and that the variety of isoforms arise from alternative splicing, glycosylation and proteolytic cleavage. Alternative splicing occurs at the 3'-terminal of the gene resulting in a precursor form of 317 and 344 amino acids[4], and then following subsequent cleavage of the 29 amino acid signal peptide, generates 2 mature follistatin isoforms of 288 and 315 amino acids (namely Follistatin-288 and Follistatin-315). The ratio of Follistatin isoforms present in different tissues and bodily fluids varies, as does their relative binding affinities. Follistatin-288 is the predominant form present in human follicular fluid, whereas the main form in serum is Follistatin-315.[5] Follistatin-288 has a greater capacity to bind and neutralize activin and is approximately 10-fold more potent than Follistatin-315 in suppressing FSH secretion from rat pituitary cells in vitro. In addition, Follistatin-288 also binds with much greater affinity to heparin sulfate proteoglycans located on the cell surface and extracellular matrix, indicating that the Follistatin-288 isoform is primarily a membrane-bound form of Follistatin, whereas Follistatin-315 is a circulating form.

### PRINCIPLE OF THE TEST

The AnshLite™ Follistatin CLIA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to follistatin antibody coated micro titer wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated follistatin 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 set, the wells are incubated with luminogenic substrate solution. In principle, the biotinylated antibody binds to the solid phase antibody-antigen complex and, in turn, binds the SHRP conjugate. Finally, the antibody-antigen and conjugate complex bound to the well is detected by addition of a luminogenic substrate (AnshLite™ chemiluminescence substrate solution). The relative light output units (RLU) measured. The absorbance measured is directly proportional to the concentration of follistatin in the samples and calibrators.

### MATERIALS SUPPLIED

**CAL-117A Follistatin Calibrators A / Sample Diluent**

One bottle, 11 mL, labeled Follistatin Cal A/Sample Diluent, containing 0 ng/mL follistatin in protein based buffer and Pro-Clean 400. Store unopened at 2-8°C until the expiration date.

**CAL-117G Follistatin Calibrators G(Lyophilized)**

Reconstitute the Follistatin Calibrator G with **1 mL** of deionized water. Solubilize, Mix well and use after reconstitution. The concentration of the calibrator F in the stock solution is approximately 20 ng/mL. Refer to the **vial label** for the exact concentration. Store unopened at 2-8°C until the expiration date.

**PLT-217 Follistatin Coated Microtitration strips**

One stripholder, containing 12 strips and 96 microtitration wells with follistatin 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-117 Follistatin Assay Buffer**

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

**BCC-217 Follistatin Biotin Conjugate Concentrate**

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

**CND-117 Follistatin 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-217 Follistatin Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)**

One amber bottle, 13 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.

**ALA-100A Anshlite™ A Solution**

One bottle, 12 mL, containing a chemiluminescent substrate solution A. Store at 2-8°C until expiration date.

**ALB-100B AnshLite™ B Solution**

One vial, 75 uL, containing an oxidizing solution B. Store at 2-8°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. 96 well, Microplate luminometer.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.

## 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.[6]

### WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide as a preservative.[7] 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 and lithium-heparin plasma 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.[8]

## PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the AnshLite™ Follistatin CLIA 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. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the substrate solution into the wells. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

## PREPARATION OF REAGENTS

**1. Follistatin Calibrators:**

a. Tap and reconstitute Follistatin stock with 1 mL deionized water. Solubilize for ten minutes, mix well before use.

b. Prepare **six** polystyrene tubes and label them as Cal A, Cal B, Cal C, Cal D, Cal E, and Cal F.

c. Add **500 µl** of Follistatin Calibrator A/Sample Diluent to each polystyrene tube labeled Cal A-F.

d. Add **500 µl** of reconstituted Follistatin Calibrator G (from step a) to the tube labeled Cal F. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

e. Add **500 µl** of Cal F (from step d) to the tube labeled Cal E. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

f. Add **500 µl** of Cal E (from step e) to the tube labeled Cal D. Vortex and mix the content in the tube thoroughly before the next dilution transfer.

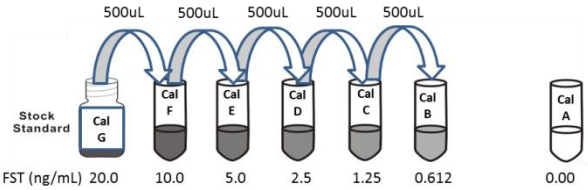
g. Add **500 µl** of Cal D (from step f) to the tube labeled Cal C. Vortex and mix the content in the tube thoroughly before use.

h. Add **500 µl** of Cal C (from step g) to the tube labeled Cal B. Vortex and mix the content in the tube thoroughly before use.

i. The tube labeled Cal A contains **500 µl** follistatin Calibrator A/Sample Diluent and has zero Follistatin concentration and should be used as **Blank**.

j. The Calibrators A-G for instance should read as 0.0 ng/mL, 0.625 ng/mL, 1.25 ng/mL, 2.5 ng/mL, 5.0 ng/mL, 10 ng/mL and 20 ng/mL. Aliquot and freeze the Follistatin Cal G Stock immediately for multiple uses. Avoid repeated freeze thaws. Frozen aliquots at -20°C are good for **one year.**

k. The Follistatin concentration in the Follistatin Calibrators is traceable to the manufacturer's working calibrators. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.




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. **Microtitration 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.
4. **Follistatin Antibody-Biotin Conjugate Solution**: The follistatin Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of follistatin 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 diluent.
5. **Substrate Solution:** Mix 1 part of AnshLite™ B in 1000 part of AnshLite™ A (for example: 12 uL of AnshLite™ B in 12 mL of AnshLite™ A). The two components should be mixed thoroughly by gentle inversion at least 60 minutes prior to use.

   ***NOTE:*** *This premixed substrate solution is stable for 8 hours at 2-8°C. Mixed substrate solution should be protected from excessive heat or direct sunlight.*

## ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

***NOTE:*** *All serum samples should be mixed and diluted (1:1) in the 0 ng/mL Calibrator A/Sample diluent prior to assay.*

1. Label the microtitration strips to be used.
2. Pipette **50 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
3. Add **50 µL** of the Follistatin Assay Buffer to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for 9**0 minutes** at room temperature (23 ± 2°C).
5. Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.
6. Add **100 µL** of the Follistatin Biotin Conjugate Solution to each well using a repeater pipette.
7. 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).
8. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
9. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
10. 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).
11. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
12. Add **100 µL** of the Substrate Solution to each well using a repeater pipette.
13. Incubate the wells, shaking at **600-800 rpm** on an orbital microplate shaker for **4±1 minute** at room temperature (23 ± 2°C). Avoid exposure to direct sunlight.
14. Read the light output of the solution in the wells **within 10 minutes**, using a microplate illuminometer.

    ***NOTE:*** *While reading the RLU'S of the well, it is necessary to program the 0 calibrator as "**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. Optimum results can be obtained at incubation temperature of **23 ± 2°C**.
2. Calculate the mean Relative Light Unit (RLU) for each Calibrator, Control, or Unknown.
3. Plot the log of the mean RLU readings for each of the Calibrators along the y-axis versus log of the follistatin concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the follistatin concentrations of the Controls and Unknowns from the calibration curve by matching their mean RLU readings with the corresponding follistatin concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A / Sample Diluent) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor.

## LIMITATIONS

The reagents supplied in this kit are optimized to measure Follistatin levels in human serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[9]

## QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- AnshLite™ Follistatin CLIA controls or other commercial controls should fall within established confidence limits.
- The Substrate solutions should be colorless. Development of any color may indicate reagent contamination or instability.

## REPRESENTATIVE CALIBRATION CURVE DATA

| Well Number | Well Contents | RLU | Conc (ng/mL) |
|---|---|---|---|
| **A1, A2** | **Calibrators** A | 0.2088 (*Blank*) | 0 |
| **B1, B2** | B | 0.161 | 0.625 |
| **C1, C2** | C | 0.519 | 1.25 |
| **D1, D2** | D | 1.795 | 2.5 |
| **E1, E2** | E | 8.255 | 5 |
| **F1, F2** | F | 31.752 | 10 |
| **G1, G2** | G | 144.409 | 20 |

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

## FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

## REFERENCES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