Activin A ELISA

RUO

AL-110

INTENDED USE

The Activin A enzyme linked immunuosorbent assay (ELISA) kit provides materials for the quantitative measurement of Activin A 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

Activin-A is a TGF-beta family member that exhibits a wide range of biological activities including regulation of cellular proliferation and differentiation, and promotion of neuronal survival. The biological activities of Activin-A can be neutralized by inhibins and by the diffusible TGF-beta antagonist, Follistatin. Human Activin-A is a 26 kDa disulfide-linked homodimer of two beta A chains, each containing 116 amino acid residues. Elevated levels of Activin-A in human colorectal tumors and in post-menopausal woman have been implicated in colorectal and breast cancers, respectively.

PRINCIPLE OF THE TEST

The Activin A ELISA is a quantitative **three-step** sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Activin A antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Activin A antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. 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 Activin A in the samples and calibrators.

MATERIALS SUPPLIED

CAL-210A Activin A Calibrators A/Sample Diluent
One vial, labeled A, containing concentration of 0 ng/mL Activin A in protein based buffer and Pro-Clean 400. Store at 2-8°C upon receipt until the expiration date.

CAL-210B - CAL-210F Activin A Calibrators B thru F
Five vials, labeled B-F, containing concentrations of approximately 0.1-10 ng/mL Activin A in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, Mix well and use after reconstitution. Aliquot and Freeze for multiple use and discard after 5 days. Avoid repeated freeze thaws. The Activin A concentration in the Activin A ELISA calibrators is traceable to WHO preparation.

CTR-210-I & CTR-210-II Activin A Controls I & II
Two vials, labeled Levels I and II containing low and high Activin A concentrations in protein based buffer with non-mercury preservative. %. Refer to **calibration card** for exact control ranges. Reconstitute Controls I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and Freeze for multiple use and discard after 5 days. Avoid repeated freeze thaws.

PLT-110 Activin A Coated Microtitration strips
One stripholder, containing 12 strips and 96 microtitration wells with Activin A 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-110A Activin A Assay Buffer A
One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

ASB-210B Activin A Assay Buffer B
One bottle, 8 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-110 Activin A 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 Activin A Biotin Conjugate diluent. Store at 2-8°C until expiration date.

CND-110 Activin A 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-110 Activin A Streptavidin-Enzyme Conjugate—Ready-to-Use (RTU)
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, 11 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, 11 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A
One bottle, 60 mL, containing phosphate buffer saline solution with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
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. Do not eat, drink or smoke where immunoassay materials are being handled. Do not pipet by mouth. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces.

b) Use personal protective equipment's. 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 kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by FDA recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. 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 and Prevention and National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[1].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400, sodium azide[2] and peroxide solution in concentrated amounts are irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Avoid contact with skin, eyes, and clothing. In case of contact with any of these reagents, wash area thoroughly with water and seek medical advice. Dispose of these reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system. 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) Use the following recommendations for handling, processing and storing blood samples.[3]

- Allow samples to clot for two hours at room temperature or overnight at 4°C and follow blood collection tube manufacturer's recommendations for centrifugation. Keep tubes stoppered at all times. Within two hours after centrifugation, transfer at least 500 µL of cell free sample to a storage tube. Tightly stopper the tube immediately.
- Samples if used within 24 hours may be stored at 4°C, otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
- Remove residual fibrin and cellular matter prior to analysis.

c) Avoid assaying lipemic, hemolyzed or icteric samples.

d) Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

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

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Activin A ELISA assay. It is 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. **Activin A Calibrators B-F and Activin A Controls I & II**: Tap and reconstitute Activin A Calibrator B-F and Activin A Controls I & II each with 1 mL deionized water. Solubilize, 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. **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. **Activin A Antibody-Biotin Conjugate Solution:** The Activin A Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Activin A Biotin 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 buffer.

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 reading higher than the highest calibrator should be mixed and diluted in the 0 ng/mL Calibrator A prior to assay.

1. Reconstitute Activin A Calibrator B-F and Activin A Controls I & II each with 1 mL deionized water. Solubilize for **10 minutes**, Mix well.
2. Label the microtitration strips to be used.
3. Pipette **25 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add **100 µL** of the Activin A Assay Buffer A to each well using a repeater pipette.
5. Add **50 µL** of the Activin A Assay Buffer B to each well using a repeater pipette.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **2 hour** at room temperature.
7. During the last 20-30 minutes of incubation, prepare the Activin A Antibody-Biotin Conjugate Solution by diluting the Activin A Biotin

Conjugate Concentrate in Activin A Biotin Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.

8. Aspirate and wash each strip **5 times** with Washing Solution using an automatic microplate washer.
9. Add **100 µL** of the Activin A Antibody-Biotin Conjugate Solution 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 **1 hour** at room temperature.
11. Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
12. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
13. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature.
14. Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
15. Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
16. Incubate the wells, shaking at on an orbital microplate shaker, for **8-12 minutes** at room temperature.
    ***NOTE**: Visually monitor the color development to optimize the incubation time.*
17. 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 microtitration well, 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 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 Activin A concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the Activin A concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Activin A concentrations.
4. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A) 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, if required.

LIMITATIONS

- The reagents supplied in this kit are optimized to measure Activin A levels in human serum.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Samples from individuals which have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interferes with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in samples[4].
- If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

QUALITY CONTROL

a) Each laboratory should establish mean values and acceptable ranges to assure proper performance.
b) Activin A ELISA controls or other commercial controls should fall within established confidence limits.
c) A full calibration curve, low and high level controls, should be included in each assay.
d) TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Optical Density	Conc (ng/mL)
	Calibrators	0.047	
A1, A2	A	(Blank)	0
B1, B2	B	0.048	0.1
C1, C2	C	0.131	0.3
D1, D2	D	0.396	1
E1, E2	E	1.151	2.9
F1, F2	F	3.037	10

***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 ng/mL.

Limit of Detection (LoD):

The lowest amount of Activin A in a sample that can be detected with a 95% probability (n=20) is 0.065 ng/mL. The value was determined by processing five samples in the range of 0.15 to 1.09 ng/mL following CLSI EP17 guidelines. Ten assay runs were performed over five days with all samples run in duplicate per run.

Limit of Quantitation (LoQ):

The estimated minimum dose achieved at 20% total imprecision is 0.07 ng/mL. The value was determined by processing six samples in the range of 0.15-1.09 ng/mL over ten runs and six days in duplicates (n=20) following CLSI EP17 guidelines.

Imprecision:

Reproducibility of the Activin A ELISA assay was determined in a study using two serum pools. The study included a total of 10 assays, four replicates of each per assay (n=40). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

Sample	Mean conc.	Within run		Between run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Pool-1	0.673	0.028	4.21%	0.026	3.83%	0.038	5.69%
Pool-2	2.527	0.107	4.25%	0.019	0.75%	0.109	4.32%

Linearity:

Based on NCCLS EP-6-P multiple dilutions of the four serum samples containing various Activin A levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	Neat	4.242	NA	NA
	1:2	2.121	2.160	102%
	1:4	1.061	1.170	110%
	1:8	0.530	0.581	110%
2	Neat	1.282	0.656	NA
	1:2	0.641	0.311	102%
	1:4	0.321	0.160	97%
	1:8	0.160		100%
3	Neat	1.842	NA	NA
	1:2	0.921	0.893	97%
	1:4	0.461	0.420	91%
4	Neat	1.243	NA	NA
	1:2	0.622	0.604	97%
	1:4	0.311	0.284	91%

Recovery:

Known amounts of Activin A were added to four serum samples containing different levels of endogenous Activin A. The concentration of Activin A was determined before and after the addition of exogenous Activin A and the percent recovery was calculated.

Sample	Endogenous Conc.(ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1	1.101	1.910	1.936	101%
		2.284	2.524	111%
2	1.36	2.145	2.268	106%
		2.509	2.676	107%
3	1.529	2.299	2.206	96%
		2.655	2.751	104%
4	1.28	2.076	2.182	105%
		2.443	2.512	103%

Analytical Specificity:

The antibody pair used in the Activin A ELISA measures total Activin A and the analyte recovery is not inhibited by the addition of follistatin 288 or 315. Other related molecules at the concentration shown below did not show any significant cross-reaction.

S.No	Cross-reactant	Concentration	% Cross-reactivity
1	Inhibin A	100 ng/mL	0.2
2	Inhibin B	100 ng/mL	ND
3	Activin B	50 ng/mL	ND
4	Activin AB	50 ng/mL	2.28
5	Alpha-2-M	100 ng/mL	ND
6	Activin-Follistatin Complex	10ng/mL	100

Interference:

When potential interferents (hemoglobin and triglycerides) were added at least at two times their physiological concentration to control sample, Activin A concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP7-P to serum matrix added.

Interferents	Analyte Conc. (mg/mL)	Unspiked Sample Value (ng/mL)	Spiked Sample Value (ng/mL)	% Difference
Hemoglobin	1.35	0.24	0.245	2.083
		2.357	2.188	-7.170
Triglygerides	5.0	0.24	0.256	6.667
		2.357	2.37	0.552

REFERENCES

1. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
2. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http:// www.cdc.gov/niosh.
3. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
4. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

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Not for use in diagnostic procedures.

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