Activin AB ELISA

RUO

AL-153

INTENDED USE

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

Activins, like all members of the transforming growth factor-beta superfamily, are synthesized as large pro-hormones with N-terminal pro- and C-terminal mature domains. Studies have shown that the prodomains template the dimerization of pro-activin forms, that are subsequently processed to yield pro/mature fragments. The prodomains, although important for folding and extracellular localization, must be removed prior to activity. Activin forms retaining the prodomain are biologically inactive. Recombinant Human Activin AB is produced from a DNA sequence encoding human Activin βA chain and human βB chain. Activin AB is expressed in CHO (Chinese Hamster Ovary) cells. Recombinant human mature Activin AB, generated by the proteolytic removal of the propeptides, is a disulfide-linked heterodimer of the mature human Activin βA chain and mature human Activin βB chain. Based on N-terminal sequencing, the βA chain starts at Gly 311 and the βB chain starts at Gly 293. The A and B monomers of recombinant human Activin AB have the same apparent molecular mass of approximately 14 kDa in SDS-PAGE under reducing conditions.

PRINCIPLE OF THE TEST

The Activin AB ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Inhibin βB subunit antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Inhibin βA subunit 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 AB in the samples and calibrators.

MATERIALS SUPPLIED

CAL-153A/Sample Diluent **Activin AB Calibrator A**

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

CAL-153B - CAL-153F **Activin AB Calibrators B thru F (Lyophilized)**

Five vials, labeled B-F, containing concentrations of approximately 1.5-110 pg/mL Activin AB in a 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 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze in plastic vials at -20°C for multiple use. Avoid repeated freeze thaws.

CTR-153-I & CTR-153-II **Activin AB Controls I & II (Lyophilized)**

Two vials, labeled Levels I and II containing low and high Activin AB concentrations in a 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 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze in plastic vials at -20°C for multiple use. Avoid repeated freeze thaws.

PLT-107 **Inhibin B Coated Microtitration Strips**

One strip holder, containing 12 strips and 96 microtitration wells with Inhibin βB subunit 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-123A **Inhibin A Assay Buffer A**

One bottle, 6 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

ASB-123B **Inhibin A Assay Buffer B**

One bottle, 6 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-153 **Activin AB Biotin Conjugate Concentrate**

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

CND-207 **Inhibin B 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-153 **Activin AB 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, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 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. Microtitration plate 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. Repeater pipette.
6. Vortex mixer.
7. 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, 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[1].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[2] 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 or follicular fluid are 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 specimens3.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Activin AB 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 (23 ± 2°C) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit components 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 AB Calibrators B-F and Activin AB Controls I & II**: Tap and reconstitute Activin AB Calibrator B-F and Activin AB 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 (23 ± 2°C) 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 AB Antibody-Biotin Conjugate Solution:** The Activin AB Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1-part conjugate to 50 parts of Inhibin B 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.

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

1. Reconstitute Activin AB Calibrator B-F and Activin AB 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 **100 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add **50 µL** of the Inhibin A Assay Buffer A to each well using a repeater pipette.
5. Add **50 µL** of the Inhibin 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 **4 hours** at room temperature (23 ± 2°C).

7. During the last **20-30 minutes** of incubation, prepare the Activin AB Antibody-Biotin Conjugate Solution by diluting the Activin AB Biotin Conjugate Concentrate in Inhibin B 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 **(350 µL/per well**) using an automatic microplate washer.
9. Add **100 µL** of the 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 (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 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 (23 ± 2°C).
14. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well**) 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 **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    ***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. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
2. Calculate the mean OD for each calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Activin AB concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the Activin AB concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Activin AB concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (CAL A) 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, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Activin AB levels in human serum and follicular fluid. 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[4].

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Activin AB ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Activin AB 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 Calibrators	Mean Absorbance	Conc (pg/mL)
A1, A2	A	0.052 (**Blank**)	0
B1, B2	B	0.107	1.35
C1, C2	C	0.158	2.7
D1, D2	D	0.411	10
E1, E2	E	1.097	34
F1, F2	F	3.052	108

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

ANALYTICSL CHARACTERISTICS

All analytical characteristics are stated in pg/mL.

Analytical Sensitivity:

The analytical sensitivity in the Activin AB ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 24 replicates of Calibrator A (0 pg/mL) and Calibrator B (1.35 pg/mL) is 0.117 pg/mL.

Imprecision:

Reproducibility of the Activin AB ELISA assay was determined in a study using two kit controls and two serum pools. The study included a total of 12 assays, four replicates of each per assay (n=48). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

Sample	Mean Conc. (pg/mL)	Within Run		Between Run		Total	
		SD	%CV	SD	%CV	SD	%CV
CI	22.180	0.597	2.69%	0.579	2.61%	0.831	3.75%
CII	68.093	2.797	4.11%	2.276	3.34%	3.606	5.30%
QC1	11.095	0.266	2.39%	0.483	4.35%	0.551	4.97%
QC2	41.767	1.818	4.35%	1.375	3.29%	2.280	5.46%

Linearity:

Multiple dilutions of Calibrator F and four follicular fluid samples containing various Activin AB levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Value in pg/mL	Observed Value in pg/mL	%Recovery
Calibrator F	Neat	108.000		
	1:2	54.000	56.190	104%
	1:4	27.000	27.690	103%
	1:8	13.500	13.260	98%
	1:16	6.750	7.210	107%
	32	3.375	3.270	97%
FF-1 (1:200)	Neat	82.750		
	1:2	41.375	44.290	107%
	1:4	20.688	21.260	103%
	1:8	10.344	10.360	100%
	1:16	5.172	4.860	94%
FF-2 (1:200)	Neat	88.800		
	1:2	44.400	47.460	107%

	1:4	22.200	23.270	105%
	1:8	11.100	11.210	101%
	1:16	5.550	5.460	98%
FF-3 (1:200)	Neat	85.020		
	1:2	42.510	44.970	106%
	1:4	21.255	21.740	102%
	1:8	10.628	10.540	99%
	1:16	5.314	5.080	96%
FF-2 (1:200)	Neat	91.228		
	1:2	45.614	46.890	103%
	1:4	22.807	23.920	105%
	1:8	11.404	11.440	100%

Recovery:

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

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	5.277	25.822 15.549	26.003 15.733	101% 101%
2	10.024	29.619 19.822	32.586 20.677	110% 104%
3	25.874	42.299 34.087	42.116 33.536	100% 98%
4	5.263	25.810 15.537	25.284 14.641	98% 94%

Analytical Specificity:

This monoclonal antibody pair used in the assay detects human, equine, canine, rabbit, goat, sheep, and mouse. Activin AB Cross-reactivity to other closely related analyte is represented in the table below.

Cross-Reactant	Concentration	% Cross-reactivity
Activin A	50 ng/mL	0.035%
Activin B	50 ng/mL	ND
FSTL3	50 ng/mL	ND
Myostatin	50 ng/mL	ND
Inhibin A	10 ng/mL	0.166%
Inhibin B	10 ng/mL	ND

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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