

Mouse/Rat Activin B ELISA

RUO

AL-156

INTENDED USE

The Mouse/Rat Activin B enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Activin B in mouse 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- β superfamily, are synthesized as large pro-hormones with N-terminal pro- and C-terminal mature domains. Studies have shown that the pro-domains template the dimerization of pro-activin forms, that are subsequently processed to yield pro/mature fragments. The pro-domains, although important for folding and extracellular localization, must be removed prior to activity. Activin forms retaining the pro-domain are biologically inactive.

Activin B, like certain other members of the TGF- β family, signals through the ActRII receptor (Activin Receptor type II). Human Activin B is a 25.6 kDa disulfide-linked homodimer consisting of two β B chains, each containing 215 amino acid residues.

Activin B exhibits a wide range of biological activities, including regulation of embryogenesis, osteogenesis, hematopoiesis, reproductive physiology and hormone secretion from the hypothalamic, pituitary and gonadal glands.

PRINCIPLE OF THE TEST

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

MATERIALS SUPPLIED

CAL-156A Mouse/Rat Activin B Calibrator A/Sample Diluent

One vial, 4 mL, labeled A, containing concentration of 0 pg/mL Activin B in protein-based buffer and ProClin™ 300. Store at 2-8°C upon receipt until the expiration date.

CAL-156B - CAL-156F Mouse/Rat Activin B Calibrators A - F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 17-2000 pg/mL Activin B in protein-based buffer and ProClin™ 300. 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 at -20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

CTR-156-I & CTR-156-II Mouse/Rat Activin B Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high Activin B concentrations in protein-based buffer and 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 at -20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

PLT-156 Activin B Coated Microtitration Strips

One strip holder, containing 12 strips and 96 microtitration wells with Activin B 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-156 Mouse/Rat Activin B 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.

BCA-156 Mouse/Rat Activin B 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 B Conjugate diluent. Store at 2-8°C until expiration date.

CND-150 Activin 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-156 Mouse/Rat Activin B 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. 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 μ L.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.
8. Tight fitting 12 x 75 mm tube racks.

WARNINGS AND PRECAUTIONS

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

The following precautions should be observed:

- a) Follow good laboratory practices.
- 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 animal and/or 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," 6th Edition, 2020¹.

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin™ 300 and Sodium azide² as a preservative. ProClin™ 300 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 Mouse Activin B 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 ($23 \pm 2^\circ\text{C}$) 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. **Mouse/Rat Activin B Calibrators B-F and Mouse/Rat Activin B Controls I & II:** Tap and reconstitute Mouse/Rat Activin B Calibrator B-F and Mouse/Rat Activin B 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 ($23 \pm 2^\circ\text{C}$) when stored in a tightly sealed bottle.
3. **Microtitation 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. **Mouse/Rat Activin B Antibody-Biotin Conjugate Solution:** The Mouse/Rat Activin B Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Mouse/Rat Activin B Conjugate Diluent, according to the number of wells used. If an entire plate is to be used, pipet exactly 220 μ L of the Concentrate into 11 mL of the diluent.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature ($23 \pm 2^\circ\text{C}$) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

Note:

- i) All mouse and rat serum samples (Unknowns) can be diluted 1 part in 4 parts (1:5) in 0 pg/mL Calibrator A/Sample Diluent prior to assay if sample volume is limited.
- ii) All serum samples reading higher than the highest calibrator should be diluted in the 0 pg/mL Calibrator A/Sample Diluent prior to assay.

1. Reconstitute Mouse/Rat Activin B Calibrator B-F and Activin B 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 **50 μ L** of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add **50 μ L** of the Activin B 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 **2 hours** at room temperature ($23 \pm 2^\circ\text{C}$).

6. During the last **20-30 minutes** of incubation, prepare the Mouse/Rat Activin B Antibody-Biotin Conjugate Solution by diluting the Mouse/Rat Activin B Biotin Conjugate Concentrate in Activin B Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
 7. Aspirate and wash each strip **5 times** with Washing Solution (**350 µL/per well**) using an automatic microplate washer.
 8. Add **100 µL** of the Antibody-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 **1 hour** at room temperature ($23 \pm 2^\circ\text{C}$).
 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 ($23 \pm 2^\circ\text{C}$).
 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 chromophore 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 **8-12 min** at room temperature ($23 \pm 2^\circ\text{C}$).
- 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: Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.

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 ($23 \pm 2^\circ\text{C}$).
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 Mouse/Rat Activin B concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the Mouse/Rat Activin B concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Mouse/Rat Activin B 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 Mouse/Rat Activin B levels in mouse serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. Avoid using icteric samples. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples⁴.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to ensure proper performance.
- Activin B ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Activin B 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 OD	Conc (pg/mL)
A1, A2	A (Blank)	0.092	0
B1, B2	B	0.033	20.0
C1, C2	C	0.063	57.5
D1, D2	D	0.251	197.0
E1, E2	E	1.227	707.0
F1, F2	F	2.714	2000.0

NOTE: CAL B – F mean absorbance data listed above is after blank subtraction.
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 pg/mL.

Limit of Detection (LoD):

The lowest amount of Activin B in a sample that can be detected with a 95% probability (n=10) is 5.28pg/mL. Ten assay runs were performed with all the samples run in duplicates.

Imprecision:

Reproducibility of the Activin B assay was determined in a study using two serum pools and two kit controls. The study included a total of 20 assays, four replicates of each per assay (n=78-80). 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	
	(pg/mL)	SD	%CV	SD	%CV	SD	%CV	
Pool-1	94.94	2.71	2.9%	5.07	5.3%	5.76	6.1%	
Control I	86.46	5.28	6.1%	3.89	4.5%	6.55	7.6%	
Pool-2	85.33	3.33	3.9%	4.80	5.6%	5.84	6.8%	
Control II	286.25	11.42	4.0%	11.66	4.1%	16.32	5.7%	

Linearity:

Based on NCCLS EP-6-P multiple dilutions of mouse samples (already diluted 1:10) containing various Mouse/Rat Activin B levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	Neat	280.28	NA	NA
	1:2	140.14	140.60	100%
	1:4	70.07	71.60	102%
	1:8	35.03	38.60	110%

2	Neat	797.594	NA	NA
	1:2	398.80	405.00	102%
	1:4	199.40	198.00	99%
	1:8	99.70	105.50	106%
	1:16	49.85	52.07	104%
	1:32	24.29	25.300	102%
3	Neat	104.90	NA	NA
	1:2	52.45	54.30	104%
	1:4	26.22	29.00	111%

Recovery:

One serum sample with a known concentration of Mouse/Rat Activin B were added to two serum samples containing different levels of endogenous Mouse/Rat Activin B. The concentration of Mouse/Rat Activin B was determined before and after the addition of exogenous Mouse/Rat Activin B and the percent recovery was calculated.

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	280.28	538.94	537.80	100%
		625.11	66.05	107%
2	280.28	192.57	157.09	82%
		263.34	131.50	81%

Analytical Specificity:

This monoclonal antibody pair used in the assay detects Mouse Activin B. Other related molecules at the concentration in the table below did not show any significant cross-reactivity. This monoclonal antibody pair is also seen to recognize other species such as Humans, Rabbit, Goat, Sheep, Bovine, Canine, Equine and Fish.

Sample	Cross-reactant	Concentration	% Cross-reactivity
1	Inhibin A	100 ng/mL	ND
2	Inhibin B	1 ng/mL	ND
3	Activin A	50 ng/mL	ND
4	Activin AB	2 ng/mL	ND
5	FST-288	50 ng/mL	ND
6	FST-315	50 ng/mL	ND
7	FSTL-3	44 ng/mL	0.1%

Interference:

When hemoglobin and triglycerides were added at a greater than two folds of their physiological concentration to control sample, Activin B concentration were within $\pm 10\%$ of the control as represented in the following table.

Interferent	Interferent Dose	Sample ID	Sample Activin B (pg/mL)	Dosed Sample Activin B (pg/mL)	% Difference
Hemoglobin	1.35 mg/mL	1	42.61	38.62	-9.4
		2	329.35	314.74	-4.4
		3	111.31	101.13	-9.1
Biotin	600 ng/mL	1	160.49	158.93	-1.0
		2	187.92	187.61	-0.2
		3	226.65	221.49	-2.3
Intralipids	10 mg/mL	1	90.13	93.08	3.3
		2	115.81	115.81	0.0
		3	125.85	123.41	-1.9
Bilirubin	0.66 mg/mL	1	150.51	164.26	9.1
		2	184.12	199.70	8.5
		3	199.06	186.34	-6.4

Inhibition Study:

Activin B complexed with each A2M, Myostatin, FST-288 and FST-315 respectively were studied in order to determine which form of Activin B is measured by the assay. It was observed that Activin B ELISA measures the total Activin B in samples.

ID	Inhibitors	Spiked Inhibitor Conc. (pg/mL)	Recovered Activin B Conc. (pg/mL)
1		0	1051.05

2	Alpha 2Microglobulin pg/mL	1000	1048.31
3		2000	1030.46
4		4000	1073.44
5		8000	1036.75
6		16000	1118.61
7		Myostatin pg/mL	1000
8	2000		998.44
9	4000		1034.72
10	8000		1064.16
11	FST-288 pg/mL	16000	1033.95
12		1000	1025.85
13		2000	988.69
14		4000	969.93
15		8000	958.13
16		16000	976.91
17	FST-315 pg/mL	1000	1034.67
18		2000	1025.24
19		4000	1017.24
20		8000	1010.60
21		16000	1015.15

REFERENCES

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