

# Ultra-Sensitive Inhibin B ELISA



## AL-195-i

### INTENDED USE

The US Inhibin B enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Inhibin B in human serum and other biological fluids. This assay is intended for in vitro diagnostic use.

### SUMMARY AND EXPLANATION

Inhibin B is a dimeric hormone that is composed of alpha ( $\alpha$ ) and beta B ( $\beta_B$ ) subunits. The free alpha subunits usually do not have any physiological effect. Therefore, the bioactivity of the inhibin's depends on the formation of a dimeric  $\alpha$ - $\beta$  structure, and only dimeric forms of inhibin's are biologically active. Inhibin's are protein hormones secreted by granulosa cells of the ovary in the female and Sertoli Cells of the testis in the male. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and have local paracrine actions in the gonads. Inhibin B levels have been reported in Sertoli Cell function (potential marker for spermatogenesis and testicular function), ovarian reserve and granulosa cell tumors.<sup>1-5</sup>

### PRINCIPLE OF THE TEST

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

### MATERIALS SUPPLIED

#### CAL-195A - CAL-195F US Inhibin B Calibrators A thru F (Lyophilized)

Six vials, labeled A-F, containing concentrations of approximately 0-550 pg/mL Inhibin B in serum and a non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators A-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use. Avoid repeated freeze thaws.

#### CTR-195-I & CTR-195-II US Inhibin B Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high Inhibin B concentrations in serum 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 immediately 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 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-195A US Inhibin B Assay Buffer A

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

#### ASB-195B US Inhibin B Assay Buffer B

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

#### BCR-195 US Inhibin B Biotin Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing Inhibin B Antibody-Biotin Conjugate in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

#### SAR-195 US Inhibin 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, 405 nm and 630 nm.
2. 96 Well Microplate orbital shaker.
3. 96 Well Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250  $\mu$ L.
5. Vortex mixer.
6. Deionized water.

## WARNINGS AND PRECAUTIONS

### For *in vitro* diagnostic use.

The following precautions should be observed:

- Follow good laboratory practice.
- Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
- Handle and dispose of all reagents and material in compliance with applicable regulations.
- If external package is damaged, inspect the components inside for any other damage. Do not use if the components are damaged.

### WARNING: Potential Biohazardous Material

This reagent may contain some human/animal 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," 5<sup>th</sup> Edition, 2007.<sup>6</sup>

### WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide<sup>7</sup> 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

- Serum and Lithium Heparin Plasma are the recommended sample types.
- 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.
- 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.
- Avoid assaying lipemic, hemolyzed or icteric samples.
- Avoid repeated freezing and thawing of samples. Thaw samples no more than 2 times.
- 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 safe handling procedures (e.g., DOT and IATA requirements) when shipping specimens.<sup>8</sup>

## PROCEDURAL NOTES

- A thorough understanding of this package insert is necessary for successful use of the US Inhibin 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.
- A calibration curve must be included with each assay.
- 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 component and do not use any component beyond the expiration date.
- 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.

- 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

- US Inhibin B Calibrators A-F and US Inhibin B Controls I & II:** Tap and reconstitute the US Inhibin B Calibrator A-F and US Inhibin B Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution.
- 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.
- 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.

## 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.

- Label the microtitration strips to be used.
- Pipette **50 µL** of the Calibrators, Controls and Unknowns to the appropriate wells.
- Add **50 µL** of the US Inhibin B Assay Buffer A to each well using a repeater pipette.
- Add **50 µL** of the US Inhibin B Assay Buffer B to each well using a repeater pipette.
- 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).
- Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.
- Add **100 µL** of the US Inhibin B Antibody-Biotin Conjugate RTU (ready-to-use) to each well using a repeater pipette.
- 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).
- Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.
- Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **15 minutes** at room temperature (23±2°C).
- Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
- Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
- 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.*
- 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.

- Optimum results can be obtained at incubation temperature of  $(23 \pm 2^\circ\text{C})$ .
- Calculate the mean OD for each Calibrator, Control, or Unknown.
- Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Inhibin B concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
- Determine the Inhibin B concentrations of the Controls and Unknowns from the calibration curve by matching their mean OD readings with the corresponding Inhibin B concentrations.
- Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (CAL A) and re-assayed.
- Any sample reading lower than the analytical sensitivity should be reported as such.
- Multiply the value by a dilution factor, if required.

**LIMITATIONS**

The reagents supplied in this kit are optimized to measure Inhibin B levels in human serum and plasma. 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.<sup>9</sup>

**QUALITY CONTROL**

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- US Inhibin B ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for US Inhibin 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	Mean OD	Conc (pg/mL)
A1, A2	Calibrators A	0.022 (Blank)	0
B1, B2	B	0.033	3.9
C1, C2	C	0.14	19.0
D1, D2	D	0.45	65.0
E1, E2	E	1.24	191.0
F1, F2	F	3.14	542.0

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

**ANALYTICAL CHARACTERISTICS****Limit of Detection (LoD):**

The Limit of detection in the assay as calculated by the interpolation of mean plus two standard deviation of 16 replicates of calibrator A (0 pg/mL) and calibrator B (3.9 pg/mL) is 0.77 pg/mL.

**Imprecision:**

Reproducibility of the US Inhibin B ELISA assay was determined on two kit controls (n=34) and four QC samples (n=40). Representative data calculated are presented in the following table.

SUMMARY:		Within run			Between run		Total	
Sample	Mean	SD	CV	SD	CV	SD	CV	
Control I	38.2	2.1	5.6%	0.0	0.0%	2.1	5.6%	
Control II	114.1	4.6	4.0%	1.3	1.1%	4.7	4.2%	
QC1	9.3	0.5	5.8%	0.4	4.6%	0.7	7.4%	
QC2	47.4	1.8	3.8%	0.0	0.0%	1.8	3.8%	
QC3	94.2	2.9	3.1%	1.5	1.6%	3.3	3.5%	
QC4	262.8	6.2	2.4%	4.0	1.5%	7.4	2.8%	

**Recovery:**

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

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	62.354	78.5	79.2	101%
		94.7	95.1	100%
		110.9	110.0	99%
2	135.642	167.2	167.2	100%
		178.7	182.6	102%
		190.2	196.4	103%
3	239.387	246.7	243.6	99%
		254.0	249.0	98%
		261.4	252.7	97%
4	176.785	187.2	183.9	98%
		197.7	196.6	99%
		208.2	210.6	101%
5	103.89	118.0	118.5	100%
		132.1	132.5	100%
		146.2	144.7	99%
6	86.06	101.1	98.6	98%
		116.1	115.9	100%
		131.0	133.0	101%

**Linearity:**

Calibrator F and three serum samples containing various Inhibin B levels were diluted with calibrator A. 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	542.0	NA	NA
	2	271.0	267.7	99%
	4	135.5	135.3	100%
	8	67.8	66.8	99%
	16	33.9	33.6	99%
	32	16.9	16.9	100%
Sample 1	Neat	315.3	NA	NA
	2	157.7	151.0	96%
	4	78.8	73.6	93%
	8	39.4	37.8	96%
	16	19.7	18.8	96%
	32	9.9	9.8	99%
Sample 2	Neat	247.1	NA	NA
	2	123.5	118.1	96%
	4	61.8	60.1	97%
	8	30.9	30.1	97%
	16	15.4	15.1	98%
	32	7.7	7.7	100%
Sample 3	Neat	339.3	NA	NA
	2	169.7	167.4	99%
	4	84.8	80.9	95%
	8	42.4	41.0	97%
	16	21.2	20.9	99%
	32	10.6	11.0	104%

**Analytical Specificity:**

This monoclonal antibody pair used in the assay detects Inhibin B. Other related molecules at the concentrations specified in the table below did not show any significant cross-reaction.

Sample	Cross-reactant	Concentration	% Cross-reactivity
1	Activin A	50 ng/mL	ND
2	Activin B	50 ng/mL	ND
3	Activin AB	50 ng/mL	ND
4	Myostatin	50 ng/mL	ND
5	Follistatin 288	50 ng/mL	ND
6	Follistatin 315	50 ng/mL	ND
7	FSTL-3	50 ng/mL	ND
8	Inhibin A (WHO 91/624)	100 ng/mL	ND

**Interference:**

When potential interferents (hemoglobin, biotin, intralipids and bilirubin) were added at specified concentrations to Control samples, Inhibin B concentrations were within  $\pm 10\%$  of the control as represented in the following table.

Interferent	Interferent Dose	Sample Inhibin B (pg/mL)	Dosed Sample Inhibin B (pg/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	90.4	82.6	-8.6
	0.5 mg/mL	90.6	87.8	-3.1
	0.1 mg/mL	92.3	91.4	-1.0
Hemoglobin	1 mg/mL	205.2	199.7	-2.7
	0.5 mg/mL	222.9	216.4	-2.9
	0.1 mg/mL	224.6	219.2	-2.4
Biotin	1200 ng/mL	82.1	82.9	1.0
	600 ng/mL	89.3	89.9	0.7
	200 ng/mL	95.4	92.7	-2.8
Biotin	1200 ng/mL	202.1	201.9	-0.1
	600 ng/mL	213	213.3	0.1
	200 ng/mL	221.5	227.2	2.6
Intralipids	20 mg/mL	88.1	85.7	-2.7
	10 mg/mL	87.7	87.2	-0.6
	5 mg/mL	88.2	89.2	1.1
Intralipids	20 mg/mL	153.9	157.4	2.2
	10 mg/mL	168.5	166.4	-1.2
	5 mg/mL	168.1	166.3	-1.1
Bilirubin	0.66 mg/mL	60.3	62.1	3.0
	0.2 mg/mL	83.3	86.1	3.3
Bilirubin	0.66 mg/mL	117.2	114.7	-2.1
	0.2 mg/mL	158.5	155.8	-1.7

**Hook Effect:**

There is no high-dose hook effect at Inhibin B concentrations up to 2700 pg/mL.

**Sample Type:**

Thirteen matched Serum, Lithium Heparin Plasma and K<sub>2</sub>-EDTA Plasma specimens were compared in US Inhibin B ELISA. Analysis of the results yielded the following Regression:

Lithium-Heparin Plasma=1.09 (Serum) + 9.4 (r=0.98)

K<sub>2</sub>-EDTA Plasma=1.13 (Serum) - 2.3 (r=0.99)

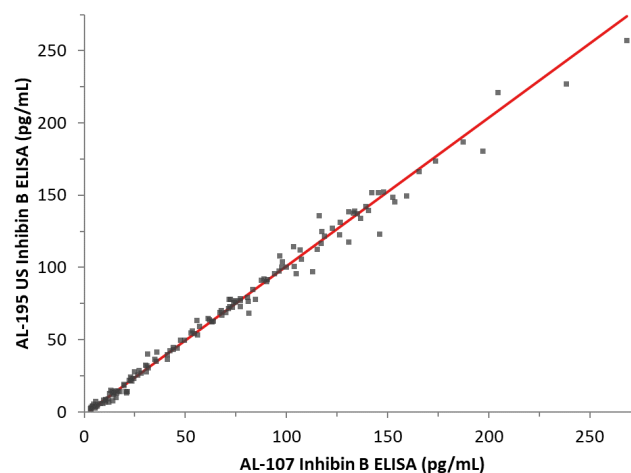
K<sub>2</sub>-EDTA Plasma=1.02 (Lithium-Heparin Plasma) – 9.2 (r=0.99)

**Method Comparison:**

The US Inhibin B ELISA (AL-195) has been compared to commercially available Inhibin B Kit (AL-107) using 140 female serum samples in the range of 3.0-268 pg/mL.

Passing Bablok analysis of the results yielded the following Regression:

US Inhibin B (AL-195) = -1.6 + 1.026 Inhibin B (AL-107), (r=0.99)

**Expected Value:**

The expected ranges for Inhibin B were calculated in serum samples using well characterized male samples (n=283) in the range of 0-72 years and female samples (n=2832) in the range of 0-76 years.

	Age Range	N	Median Age	Median Inhibin B (pg/mL)	Reference Interval* Inhibin B (pg/mL)
Males	< 15 Days	21	3 Days	158.0	68 - 373
	15-180 Days	31	66 Days	238.0	42 - 516
	0-6-7 Years	31	1.28 Years	110.0	24 - 300
	8-30 Years	98	20 Years	184.5	47 - 383
Females	31-72 Years	102	39 Years	137.0	10 - 357
	1-12 Years	65	0.6 Years	25	1 - 182
	13-41 Years**	162	27.8 Years	78.4	7.9 - 223
	42-51 Years**	35	47 Years	26.3	1 - 107
	14-47 Years***	1883	28.5 Years	103.7	6 - 359
	14-44 Years****	631	26.6 Years	103.2	10 - 266
	51-76 Years#	56	62 Years	1.0	1 - 11

\*98th Percentile, \*\* Regular Cycle (Follicular Phase), \*\*\* Oligomenorrhea, \*\*\*\* Amenorrhea, #Post-Menopausal

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Please do not use the product for exact specification

**Symbols used with Ansh Labs Assays**

Symbol	English	Deutsch	Français	Español	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	Caution	Vorsicht	mise en garde	precaución	attenzione
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	fecha de caducidad	Data di scadenza
	Date of Manufacture	Herstellungsdatum	date de fabrication	fecha de manufactura	Data di produzione
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
	96-well plate	Platte mit 96 Vertiefungen	Plaque à 96 puits	placa de 96 pocillos	piastra a 96 pozzetti

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Refer to package insert included with  
the product for exact specification