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Inhibin B Gen II ELISA

Instruction for use in local language is available at beckmancoulter.com/techdocs.

REVISION HISTORY

Previous version:	**Current version:**
Chapter INTENDED USE removed	Chapter INTENDED PURPOSE added
—	Chapter APPENDIX: Interference data added
—	CLSI guidelines incorporated

REF A81303

FOR PROFESSIONAL USE ONLY

INTENDED PURPOSE

Inhibin B Gen II ELISA is an in vitro diagnostic manual medical device intended to be used by healthcare professionals for the quantitative measurement of inhibin B in human serum and plasma. Measurement of inhibin B is intended to be used as an aid in diagnosis and monitoring of non-epithelial ovarian cancer.

PRINCIPLE

The enzyme immunoassay of inhibin B is an enzymatically amplified three-step sandwich-type assay. Mouse monoclonal antibodies directed against two different epitopes of inhibin B and hence not competing are used.

Samples and calibrators are first incubated in wells coated with the monoclonal antibody. After the first incubation, the contents of the wells are rinsed and the wells are incubated with biotinylated detection antibody. After the second incubation, the contents of the wells are rinsed and the wells are incubated with streptavidin labeled with the enzyme horseradish peroxidase (HRP). After the third incubation the contents of the wells are rinsed and the wells are incubated with the substrate tetramethylbenzidine (TMB). The bound enzymatic activity is then measured after the addition of a chromogenic substrate. The inhibin B concentrations in the samples are obtained by interpolation from the standard curve. The concentration of inhibin B in the samples is directly proportional to the absorbance.

WARNING AND PRECAUTIONS

General remarks:

- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Do not mix the reagents from kits of different lots.
- A standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Each well must be used only once.
- Incomplete washing will adversely affect the outcome and assay precision.
- Avoid microbial contamination of reagents, especially of the conjugate and the assay buffer.
- Avoid contamination of the TMB chromogen solution with the conjugates.
- For dispensing sulfuric acid and TMB chromogen solution, avoid pipettes with metal parts.
- 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.

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Dispose of the reagents by flushing with large amounts of water through the plumbing system.

Materials of human origin

All serum and plasma samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

GHS HAZARD CLASSIFICATION

Assay Buffer WARNING

	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Alcohol, C12-14-secondary, ethoxylated 1 - < 3%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Biotin Conjugate Diluent	WARNING	
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Alcohol, C12-14-secondary, ethoxylated 1 - < 3%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
AB Biotin Conjugate Concentrate	DANGER	
		
		
	H316	Causes mild skin irritation.
	H317	May cause an allergic skin reaction.
	H318	Causes serious eye damage.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.

	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Alcohol, C12-14-secondary, ethoxylated 3 - 6%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Streptavidin Conjugate RTU	DANGER	
		
	H226	Flammable liquid and vapour.
	H302	Harmful if swallowed.
	H313	May be harmful in contact with skin
	H370	Causes damage to organs.
	P210	Keep away from heat, hot surfaces, and sparks. No smoking.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P311	If exposed or concerned: Call a doctor/physician.
	P312	Call a POISON CENTER or doctor/physician if you feel unwell.
		Methanol 1 - 9%
Stopping Solution A	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Sulfuric Acid 1 - 3%
Wash Solution (20X) for "CZ" lot only	DANGER	
		
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.

Boric Acid 0.1 - 0.3%
Sodium Borate Decahydrate 0.1 - 0.3%

SDS Safety Data Sheet is available at beckmancoulter.com/techdocs

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- Serum and lithium heparin plasma are the recommended sample types.
- Allow serum samples to clot completely before centrifugation.
- Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
- Serum and plasma samples may be stored tightly stoppered at 2-8°C, if the assay is to be performed within 48 hours. For longer storage keep frozen (at < -20°C), after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.
- If samples have concentrations greater than the highest calibrator, they must be diluted into the zero calibrator.

Use the following guidelines when preparing samples:

- Ensure residual fibrin and cellular matter have been removed prior to analysis.
- Follow blood collection tube manufacturer's recommendations for centrifugation.

Serum and lithium heparin plasma values for 120 samples (serum values ranging from 0.00 to 556.6 pg/mL) were compared using the Inhibin B Gen II ELISA. Results are as follows: [Li-Hep plasma] = 0.972[serum]+0.000; R = 0.995

CONTENTS

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76). To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Storage conditions for reagents after dilution are indicated in paragraph Procedure.

Plate: 12 x 8 wells (ready-to-use)

Unused strips have to be stored at 2-8°C in the self-lock bag provided.

Antibody-Biotin conjugate concentrate: one 0.4 mL vial

The vial contains a solution of biotinylated anti-inhibin α-subunit antibody in buffer with protein (mouse), ProClin* 300 (<0.5%).

Streptavidin-Enzyme conjugate: one 13.0 mL bottle (ready-to-use)

The bottle contains conjugated HRP in buffer with protein (fish) and methanol (<10%).

Assay buffer: one 8 mL bottle (ready-to-use)

The bottle contains buffer with bovine serum albumin (BSA), protein (bovine, mouse, goat), surfactant, and ProClin* 300 (<0.5%).

Biotin conjugate diluent: one 13 mL bottle (ready-to-use)

The bottle contains buffer with BSA, protein (bovine, mouse, goat), surfactant and ProClin* 300 (<0.5%).

TMB Chromogen solution: one 11 mL bottle (ready-to-use)

The bottle contains a solution of TMB in citrate buffer with hydrogen peroxide.

TMB Chromogen solution (11 mL) may be ordered separately, too (cat. #DSL-10-9755-1).

Wash solution U (20X): one 50 mL vial

Concentrated solution has to be diluted before use. It may be ordered separately, too (cat. #A54825).

Stopping solution A: one 11 mL bottle (ready-to-use)

The bottle contains 0.2 M sulfuric acid.

Stopping solution A (11 mL) may be ordered separately, too (cat. #C24811).

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- **Inhibin B Gen II Calibrators and Controls, supplied upon REF. A81304.**
- Orbital microtiter plate shaker.
- Precision pipette to deliver 10–1,000 µL.
- Microtiter plate washer (optional).
- Vortex type mixer.

- Absorbent material for blotting strips.
- Microtiter plate reader (450/405 nm and 600-630 nm) (bichromatic reading).

PROCEDURE

Preparation of reagents

Let all the reagents come to room temperature and mix them thoroughly by gentle inversion before the use.

Preparation of the wash solution

Pour the content of the vial into 950 mL of distilled water and homogenize. The diluted solution can be stored in a tightly sealed bottle at 18-25°C one month or at 2-8°C until the expiry date of the kit.

Preparation of the Antibody-Biotin conjugate concentrate

The Antibody-Biotin conjugate concentrate should be freshly diluted 10-30 minutes before use. For an entire plate, pipette exactly 220 µL of the Antibody-Biotin conjugate concentrate into 11 mL of the Biotin conjugate diluent. The Antibody-Biotin conjugate concentrate should be diluted at a ratio of 1 part into 50 parts of Biotin conjugate diluent, according to the number of wells used.

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

Step 1 Additions, 1st incubation	Step 2 2nd incubation	Step 3 3rd incubation
To coated wells add successively: 50 µL of calibrator, control or sample and 50 µL of Assay buffer. Incubate 2 hours at 18-25°C with shaking (600-800 rpm).	Aspirate carefully the contents of each well. Wash 5 times with 350 µL of the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material. Add 100 µL of Antibody-Biotin conjugate solution to each well. Incubate 1 hour at 18-25°C with shaking (600-800 rpm).	Aspirate carefully the contents of each well. Wash 5 times with 350 µL of the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material. Add 100 µL of Streptavidin-Enzyme conjugate solution to each well. Incubate 30 minutes at 18-25°C with shaking (600-800 rpm).

Step 4 Enzymatic step	Step 5 Reading
Aspirate carefully the contents of each well. Wash 5 times with 350 µL of the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material. Add 100 µL of chromogenic substrate to each well.* Incubate 8-12 minutes at 18-25°C with shaking (600-800 rpm).**	Add 100 µL of stop solution to each well.*** Read absorbance within 30 minutes at 450 nm.****

**Avoid exposure to direct sunlight.*

***Be aware that color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Visually monitor the color development to optimize the incubation time.*

****To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB chromogenic solution.*

*****If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set between 600-630 nm.*

RESULTS

Results are obtained from the calibrator curve by interpolation. The curve serves for the determination of analyte concentrations in samples measured at the same time as the calibrators.

Standard curve

The results in the quality control department were calculated using *weighted cubic regression* curve fit with ABS on the log vertical axis and analyte concentration of the calibrators on the log horizontal axis.

Other calculation methods may give slightly different results.

Calibrators	Inhibin B (pg/mL)	ABS	ABS/ABS_{max} (%)
0	0	0.042 (blank)	-
1	11	0.081	2.60

Calibrators	Inhibin B (pg/mL)	ABS	ABS/ABS_{max} (%)
2	33	0.166	5.32
3	110	0.460	14.8
4	275	0.995	31.9
5	550	1.744	55.9
6	1,100	3.118	100

ABS = Absorbance
(Example of standard curve, do not use for calculation).

Samples

For each sample, locate ratio ABS on the vertical axis and read off the corresponding analyte concentration on the horizontal axis.

EXPECTED VALUES

We recommend each laboratory to establish its own reference values. The following values obtained from healthy subjects are indicative only.

Serum samples were procured either from vendors or were aliquots stored in freezers from our previous studies. These samples were analyzed using the Inhibin B Gen II kit on site. The expected ranges for inhibin B were calculated using 85-95% non-parametric estimation using Analyse-it** for Microsoft Excel.***

Samples	n	Median age (years)	Median (pg/mL)	2.5-97.5th Percentile (pg/mL)
Random males	235	35	166	25-325
Random females	95	30	47	ND-341
Females 3rd day of cycle	106	NA	75	ND-273
Post menopausal females†	74	74	ND	ND-4
Boys††	15	11	93	4-352
Girls	15	11	18	ND-83

ND = Non-Detectable
†90% non-parametric
††85% non-parametric

QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com

PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Sensitivity

Limit of detection (LoD): 5.79 pg/mL

The LoD of the assay is 5.79 pg/mL, determined consistent with guidelines in CLSI document EP17-A2 [1] based on the proportions of false positives (α) less than 5% and false negatives (β) less than 5%; using determinations, with 168 blank and 168 low level samples; and Limit of Blank (LoB) of 1.83 pg/mL.

Specificity

The highly characterized antibody pair used in the assay measures 100% inhibin B in human, monkey and rat. The following potential cross reactants (inhibin A, activin A, activin B, activin AB, AMH, FSH, LH and Follistatin 315) were added at least at two times their physiological concentration to serum matrix and assayed. All inhibin B values obtained in the presence of each cross reactant were non-detectable.

Precision

Repeatability and within-laboratory precision

The precision of the assay was determined consistent with guidelines in CLSI document EP05-A3 [2]. For repeatability the coefficients of variation were found below or equal to 12.8 % for serum samples. For within-laboratory precision the coefficients of variation were found below or equal to 14.3 % for serum samples.

Accuracy

Linearity

The assay demonstrated to be linear from 5.88 to 1,264 pg/mL using serum samples (determined consistent with guidelines in CLSI document EP06-A [3]).

Dilution test

High-concentration samples were serially diluted with the zero calibrator. The recovery percentages obtained were between 92.9% and 110%.

Recovery test

Low-concentration samples were spiked with known quantities of Inhibin B. The recovery percentages obtained were between 94.5% and 105%.

Measurement range (from LoD to the highest calibrator): 5.79 to approximately 1,000 pg/mL.

LIMITATIONS

Failure to follow these instructions for use (IFU) may significantly affect results.

Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.

Do not use hemolyzed, lipemic or icteric samples. For more details, see Appendix, § Interference.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who 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 interfere with immunoassays. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies [4,5].

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

ADDITIONAL INFORMATION

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Summary

Inhibins are heterodimeric polypeptide hormones. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and also have local paracrine actions on the gonads [6,7]. The fully processed form of the inhibin molecule has a molecular weight of approximately 32-36 kD and consists of the two distinct chains (α and β), linked by disulfide bridges. Higher molecular weight forms, with precursor forms of the α-subunit, also occur in follicular fluid and serum. In addition, free α-subunit forms, unassociated with a β-subunit, and lacking inhibin bioactivity, are also present [8,9,10,11].

Inhibin B consists of an α-subunit and a β-subunit. Inhibin B is produced by the sertoli cells of the testis in the male and the granulosa cells of the ovary in the female. Its primary role appears to be in the regulation of gametogenesis via negative feedback on the production of FSH. Several published reports indicate the utility of measurement of inhibin B as an endocrine marker for monitoring the male [12,13,14,15,16,17] and female [18,19,20,21,22,23,24,25,26] gonadal function.

The Inhibin B Gen II ELISA uses the highly characterized pair of antibodies that specifically recognize only the functional dimeric inhibin B molecule and does not measure the free α-subunit forms present in biological fluids [27]. The current assay does not require sample pre-treatment step with hydrogen peroxide to oxidize two methionines in the epitope to the sulfoxide for full immunoreactivity.

Interference

Serum samples containing inhibin B concentrations (low and high) were spiked with multiple concentrations of the substances listed below and assayed using Inhibin B Gen II ELISA. Values were calculated as described in CLSI EP07, 3rd ed. [28]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Acetylsalicylic acid	48.1 µg/mL
Ascorbic acid	58.7 µg/mL
Biotin	1,694 ng/mL
Conjugated bilirubin	443 µg/mL
Hemoglobin	10,013 µg/mL
Heparin	8,003 ng/mL
Cholesterol	4.52 mg/mL
Ibuprofen	340 µg/mL
Prednisone	117 ng/mL
Prednisolone	1,343 ng/mL
Rheumatoid factor	37.0 IU/mL
Triglycerides	16.4 mg/mL
Unconjugated Bilirubin	200 µg/mL

In spite of hemoglobin, bilirubin (conjugated, unconjugated) and triglyceride interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples.

Precision

Repeatability and within-laboratory precision

Samples were assayed for 20 days, 2 runs per day, in triplicates per run. Assays were performed by two lab technicians, by two reagent lots. There were 120 individual measurements per sample with no invalid results.

Serum samples	Mean (pg/mL)	Repeatability		Within-laboratory precision	
		SD (pg/mL)	C.V. (%)	SD (pg/mL)	C.V. (%)
S1	20.1	2.57	12.8	2.84	14.2
S2	29.0	3.04	10.5	3.67	12.6
S3	111	5.21	4.68	11.7	10.6
S4	235	18.1	7.70	27.4	11.7
S5	546	48.8	8.95	62.7	11.5
S6	898	66.1	7.36	128	14.3

Li-Hep plasma samples	Mean (pg/mL)	Repeatability		Within-laboratory precision	
		SD (pg/mL)	C.V. (%)	SD (pg/mL)	C.V. (%)
P1	50.3	3.69	7.34	4.08	8.12
P2	93.6	5.53	5.91	6.47	6.91
P3	158	6.97	4.40	8.86	5.60
P4	425	44.7	10.5	44.7	10.5
P5	658	40.4	6.14	47.7	7.25
P6	989	43.5	4.40	62.0	6.27

Accuracy

Linearity

The assay demonstrated to be linear from 2.25 to 1,153 pg/mL using lithium heparin plasma samples (determined consistent with guidelines in CLSI document EP06-A [3]).

Dilution test

Samples were diluted in zero calibrator and assayed according to the assay procedure of the kit.

Li-Hep plasma sample	Dilution factor	Inhibin B (pg/mL)		Ratio (%) Measured/ Expected
		Measured	Expected	
P1	-	743.3	-	-
	1:2	359.1	371.7	96.63
	1:4	188.6	185.8	101.5
	1:8	93.85	92.91	101.0
	1:16	47.77	46.46	102.8
P2	-	676.0	-	-
	1:2	312.6	338.0	92.49
	1:4	159.9	169.0	94.62
	1:8	85.21	84.50	100.8
	1:16	42.82	42.25	101.4
P3	-	1158	-	-
	1:2	578.3	579.0	99.87
	1:4	283.6	289.5	97.97
	1:8	137.7	144.8	95.11
	1:16	60.04	72.38	82.95

Serum samples	Dilution factor	Inhibin B (pg/mL)		Ratio (%) Measured/ Expected
		Measured	Expected	
S1	-	1,032	-	-
	1:2	552.4	515.8	107.1
	1:4	284.2	257.9	110.2
	1:8	140.4	128.9	108.9
	1:16	66.17	64.47	102.6
S2	-	701.2	-	-
	1:2	344.4	350.6	98.24
	1:4	176.6	175.3	100.8
	1:8	88.79	87.66	101.3
	1:16	42.39	43.83	96.72
S3	-	606.7	-	-
	1:2	300.8	303.4	99.15
	1:4	145.9	151.7	96.20
	1:8	74.57	75.84	98.32
	1:16	35.24	37.92	92.93

Recovery test

Samples were spiked with known quantities of inhibin B and assayed according to the assay procedure of the kit.

Serum samples	Endog. conc. (pg/mL)	Added conc. (pg/mL)	Expected conc. (pg/mL)	Measured conc. (pg/mL)	Ratio (%) Measured/ Expected
S1	72.44	17.33	89.77	91.49	101.9
	73.68	53.19	126.9	129.8	102.3
	70.58	101.4	171.9	180.1	104.7
S2	45.82	12.32	58.13	59.43	102.2
	46.37	37.68	84.05	83.87	99.78
	44.96	73.06	118.0	123.9	105.0
S3	27.05	7.519	34.57	35.93	103.9
	25.69	21.55	47.24	46.31	98.03
	26.74	44.93	71.67	70.87	98.89
S4	63.97	21.31	85.28	86.27	101.2
	65.36	65.09	130.5	127.7	97.92
	62.84	108.8	171.7	166.9	97.24
S5	47.45	15.17	62.62	62.85	100.4
	48.18	46.04	94.22	88.99	94.45
	46.40	88.67	135.1	129.6	95.94

Li-Hep plasma sample	Endog. conc. (pg/mL)	Added conc. (pg/mL)	Expected conc. (pg/mL)	Measured conc. (pg/mL)	Ratio (%) Measured/ Expected
P1	19.63	6.87	26.50	28.61	108.0
	18.54	19.36	37.91	40.19	106.0
	19.45	41.23	60.68	55.98	92.26
P2	25.50	7.52	33.02	33.43	101.2
	23.95	21.10	45.06	46.78	103.8
	25.23	45.67	70.89	66.64	94.00
P3	48.28	14.07	62.34	63.78	102.3
	49.29	43.70	92.99	84.85	91.24
	47.36	83.97	131.3	114.5	87.18
P4	60.11	17.59	77.70	79.46	102.3
	61.71	54.92	116.6	117.8	101.0
	59.13	97.44	156.6	141.3	90.25
P5	51.62	15.39	67.01	66.41	99.10
	52.83	47.63	100.5	93.24	92.81
	50.56	91.65	142.2	132.9	93.48

Method comparison

The Inhibin B Gen II ELISA (A81303) has been compared to two commercially available assays (Oxford Bio-Innovation (OBI) and Diagnostics Systems Laboratories (DSL)) using 60 serum male and 60 serum female samples, ranging in age from 20–50 years. Linear regression analysis of the results yielded the following:

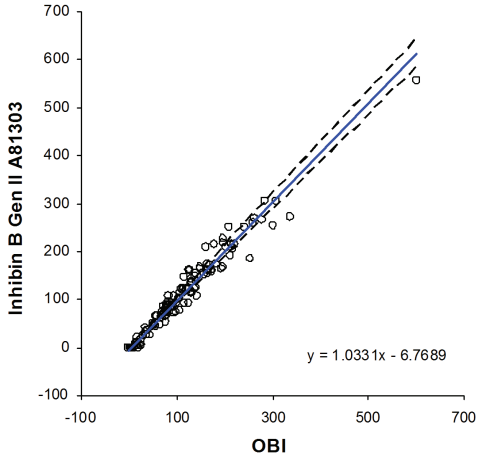
Regression using n = 120 serum samples:

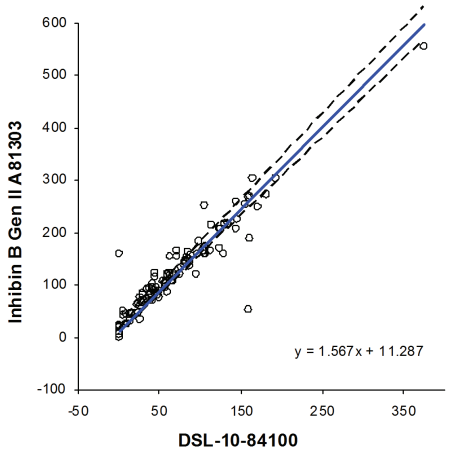
Inhibin B Gen II ELISA = 1.03 (OBI) - 6.77 pg/mL

(r = 0.99; P < 0.0001)

Inhibin B Gen II ELISA = 1.57 (DSL-10-84100) + 11.29 pg/mL

(r = 0.97; P < 0.0001)





APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Summary

Inhibins are heterodimeric polypeptide hormones. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and also have local paracrine actions on the gonads [6,7]. The fully processed form of the inhibin molecule has a molecular weight of approximately 32-36 kD and consists of the two distinct chains (α and β), linked by disulfide bridges. Higher molecular weight forms, with precursor forms of the α-subunit, also occur in follicular fluid and serum. In addition, free α-subunit forms, unassociated with a β-subunit, and lacking inhibin bioactivity, are also present [8,9,10,11].

Inhibin B consists of an α-subunit and a β-subunit. Inhibin B is produced by the sertoli cells of the testis in the male and the granulosa cells of the ovary in the female. Its primary role appears to be in the regulation of gametogenesis via negative feedback on the production of FSH. Several published reports indicate the utility of measurement of inhibin B as an endocrine marker for monitoring the male [12,13,14,15,16,17] and female [18,19,20,21,22,23,24,25,26] gonadal function.

The Inhibin B Gen II ELISA uses the highly characterized pair of antibodies that specifically recognize only the functional dimeric inhibin B molecule and does not measure the free α-subunit forms present in biological fluids [27]. The current assay does not require sample pre-treatment step with hydrogen peroxide to oxidize two methionines in the epitope to the sulfoxide for full immunoreactivity.

Interference

Serum samples containing inhibin B concentrations (low and high) were spiked with multiple concentrations of the substances listed below and assayed using Inhibin B Gen II ELISA. Values were calculated as described in CLSI EP07, 3rd ed. [28]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Acetylsalicylic acid	48.1 µg/mL
Ascorbic acid	58.7 µg/mL
Biotin	1,694 ng/mL
Conjugated bilirubin	443 µg/mL
Hemoglobin	10,013 µg/mL
Heparin	8,003 ng/mL
Cholesterol	4.52 mg/mL
Ibuprofen	340 µg/mL
Prednisone	117 ng/mL
Prednisolone	1,343 ng/mL
Rheumatoid factor	37.0 IU/mL
Triglycerides	16.4 mg/mL
Unconjugated bilirubin	200 µg/mL

In spite of hemoglobin, bilirubin (conjugated, unconjugated) and triglyceride interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples.

Precision

Repeatability and within-laboratory precision

Samples were assayed for 20 days, 2 runs per day, in triplicates per run. Assays were performed by two lab technicians, by two reagent lots. There were 120 individual measurements per sample with no invalid results.

Serum samples	Mean (pg/mL)	Repeatability		Within-laboratory precision	
		SD (pg/mL)	C.V. (%)	SD (pg/mL)	C.V. (%)
S1	20.1	2.57	12.8	2.84	14.2
S2	29.0	3.04	10.5	3.67	12.6
S3	111	5.21	4.68	11.7	10.6
S4	235	18.1	7.70	27.4	11.7
S5	546	48.8	8.95	62.7	11.5
S6	898	66.1	7.36	128	14.3

Li-Hep plasma samples	Mean (pg/mL)	Repeatability		Within-laboratory precision	
		SD (pg/mL)	C.V. (%)	SD (pg/mL)	C.V. (%)
P1	50.3	3.69	7.34	4.08	8.12
P2	93.6	5.53	5.91	6.47	6.91
P3	158	6.97	4.40	8.86	5.60
P4	425	44.7	10.5	44.7	10.5
P5	658	40.4	6.14	47.7	7.25
P6	989	43.5	4.40	62.0	6.27

Accuracy

Linearity

The assay demonstrated to be linear from 2.25 to 1,153 pg/mL using lithium heparin plasma samples (determined consistent with guidelines in CLSI document EP06-A [3]).

Dilution test

Samples were diluted in zero calibrator and assayed according to the assay procedure of the kit.

Serum samples	Dilution factor	Inhibin B (pg/mL)		Ratio (%) Measured/ Expected
		Measured	Expected	
S1	-	1,032	-	-
	1:2	552.4	515.8	107.1
	1:4	284.2	257.9	110.2
	1:8	140.4	128.9	108.9
	1:16	66.17	64.47	102.6
S2	-	701.2	-	-
	1:2	344.4	350.6	98.24
	1:4	176.6	175.3	100.8
	1:8	88.79	87.66	101.3
	1:16	42.39	43.83	96.72
S3	-	606.7	-	-
	1:2	300.8	303.4	99.15
	1:4	145.9	151.7	96.20
	1:8	74.57	75.84	98.32
	1:16	35.24	37.92	92.93

Li-Hep plasma sample	Dilution factor	Inhibin B (pg/mL)		Ratio (%) Measured/ Expected
		Measured	Expected	
P1	-	743.3	-	-
	1:2	359.1	371.7	96.63
	1:4	188.6	185.8	101.5
	1:8	93.85	92.91	101.0
	1:16	47.77	46.46	102.8
P2	-	676.0	-	-
	1:2	312.6	338.0	92.49
	1:4	159.9	169.0	94.62
	1:8	85.21	84.50	100.8
	1:16	42.82	42.25	101.4
P3	-	1158	-	-
	1:2	578.3	579.0	99.87
	1:4	283.6	289.5	97.97
	1:8	137.7	144.8	95.11
	1:16	60.04	72.38	82.95

Recovery test

Samples were spiked with known quantities of inhibin B and assayed according to the assay procedure of the kit.

Serum samples	Endog. conc. (pg/mL)	Added conc. (pg/mL)	Expected conc. (pg/mL)	Measured conc. (pg/mL)	Ratio (%) Measured/ Expected
S1	72.44	17.33	89.77	91.49	101.9
	73.68	53.19	126.9	129.8	102.3
	70.58	101.4	171.9	180.1	104.7
S2	45.82	12.32	58.13	59.43	102.2
	46.37	37.68	84.05	83.87	99.78
	44.96	73.06	118.0	123.9	105.0
S3	27.05	7.519	34.57	35.93	103.9
	25.69	21.55	47.24	46.31	98.03
	26.74	44.93	71.67	70.87	98.89
S4	63.97	21.31	85.28	86.27	101.2
	65.36	65.09	130.5	127.7	97.92
	62.84	108.8	171.7	166.9	97.24
S5	47.45	15.17	62.62	62.85	100.4
	48.18	46.04	94.22	88.99	94.45
	46.40	88.67	135.1	129.6	95.94

Li-Hep plasma sample	Endog. conc. (pg/mL)	Added conc. (pg/mL)	Expected conc. (pg/mL)	Measured conc. (pg/mL)	Ratio (%) Measured/ Expected
P1	19.63	6.87	26.50	28.61	108.0
	18.54	19.36	37.91	40.19	106.0
	19.45	41.23	60.68	55.98	92.26
P2	25.50	7.52	33.02	33.43	101.2
	23.95	21.10	45.06	46.78	103.8
	25.23	45.67	70.89	66.64	94.00
P3	48.28	14.07	62.34	63.78	102.3
	49.29	43.70	92.99	84.85	91.24
	47.36	83.97	131.3	114.5	87.18
P4	60.11	17.59	77.70	79.46	102.3
	61.71	54.92	116.6	117.8	101.0
	59.13	97.44	156.6	141.3	90.25
P5	51.62	15.39	67.01	66.41	99.10
	52.83	47.63	100.5	93.24	92.81
	50.56	91.65	142.2	132.9	93.48

Method Comparison

The Inhibin B Gen II ELISA (A81303) has been compared to two commercially available assays (Oxford Bio-Innovation (OBI) and Diagnostics Systems Laboratories (DSL)) using 60 serum male and 60 serum female samples, ranging in age from 20–50 years. Linear regression analysis of the results yielded the following:

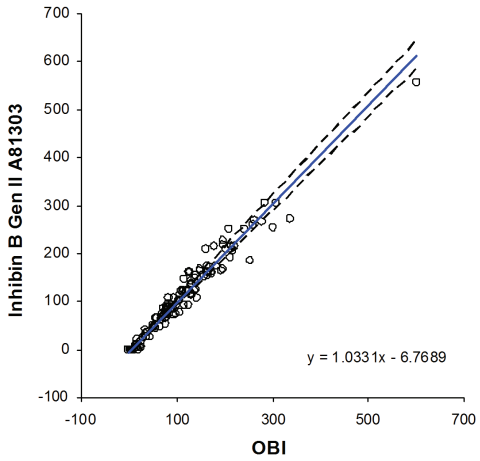
Regression using n = 120 serum samples:

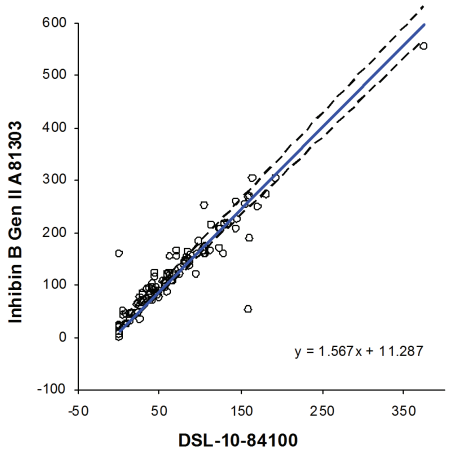
Inhibin B Gen II ELISA = 1.03 (OBI) - 6.77 pg/mL

(r = 0.99; P < 0.0001)

Inhibin B Gen II ELISA = 1.57 (DSL-10-84100) + 11.29 pg/mL

(r = 0.97; P < 0.0001)





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BUF ASSAY	Assay Buffer/ Tampon pour le dosage / Assay-Puffer / Tampone per le analisi / Tampón de análisis / Tampão de ensaio / Analysebuffer / Assay Buffert / Määrityspuskuri / Ρυθμιστικό διάλυμα ανάλυσης / Assay puffer / Bufor do testu / Tlumivý roztok / Test Tamponu / Буфер для анализа / Pufer za testiranje / Буфер за анализи / Soluţie tampon analiză / Test pufer
BIO CONJ CONC	Biotin Conjugate Concentrate / Concentré de conjugué biotine / Biotinkonjugat-Konzentrat / Concentrato del coniugato biotina / Concentrado de conjugado de biotina / Concentrado Conjugado de Biotina / Bioaktivt konjugatkoncentrat / Biotinkonjugat, koncentrat / Biotiinikonjgaatti, konsentraatti / Συμπύκνωμα συζεύγματος βιοτίνης / Biotin konjugátum koncentrátum / Koncentrat koniugatu biotyny / Koncentrát konjugátu biotinu / Biotin Konjugat Konsantresi / Конъюгат биотина, концентрат / Koncentrat konjugata biotina/ Концентрат на конюгат биотин / Concentrat conjugat biotină / Koncentrat biotin konjugata / Biotine conjugaatconcentraat
STREP CONJ RTU	Streptavidin Conjugate Concentrate/ Concentré de conjugué streptavidine/ Streptavidinkonjugat- Konzentrat/ Concentrato del coniugato streptavidina / Concentrado de conjugado de Streptavidina / Concentrado Conjugado de Enzima-Estreptavidina / Streptavidin konjugatkoncentrat / Streptavidinkonjugat, koncentrat / Streptavidiinikonjugaatti, konsentraatti /Συμπύκνωμα συζεύγματος στρεπταβιδίνης / Sztreptavidin konjugátum koncentrátum / Stężony roztówr koniugatu streptawidyny / Koncentrát streptavidinového konjugátu / Streptavidin Konjugat Konsantresi / Стрептавидин концентрат/ Koncentrat konjugata streptavidina / Концентрат на конюгат стрептавидин/ Concentrat conjugat streptavidină/ Koncentrat streptavidin konjugata/ Streptavidine conjugaatconcentraat
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SOLN WASH 20x	Wash Solution/ Solution de lavage/Waschlösung / Soluzione di lavaggio / Solución de lavado / Solução de lavagem / Vaskeopløsning / Tvättlösning / Pesuliuos / Πλυστικό υγρό / Mosó oldat / Roztwór myjący / Promývací roztok / Yıkama solüsyonu / Промывающий раствор / Otopina za ispiranje / Промиващ разтвор / Soluţie de spălare / Rastvor za pranje / Spoeloplossing
PLATE	Plate / Plaque / Platte / Piastra / Placa / Plade / Platta / Levy / Πλάκα / Lemez / Płytka / Deska / Plaka / Планшет/ Ploča / Чашка / Placă / Pločica / Plaat
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