Inhibin B GEN II ELISA

REF A81301

TABLE OF CONTENTS

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Inhibin B GEN II ELISA

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FOR PROFESSIONAL USE ONLY

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.
The Inhibin B Gen II enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of inhibin B in human serum and lithium heparin plasma.

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[1,2] 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.[3,4,5,6]

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[7,8,9,10,11,12] and female[13,14,15,16,17,18,19,20,21] 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.[22] 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.

PRINCIPLE

The Inhibin B Gen II ELISA is an enzymatically amplified three-step "sandwich" assay. In the assay, calibrators, controls and samples are incubated in microtitration wells which have been coated with anti-activin B antibody.[22] After incubation and washing, the wells are incubated with biotinylated anti-Inhibin α- subunit detection antibody. After a second incubation and washing step, the wells are incubated with streptavidin labelled with the enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB).

After incubation an acidic stopping solution is added. 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 primary reference filter. The absorbance measured is directly proportional to the concentration of inhibin B in the samples. A set of Inhibin B Gen II calibrators is used to plot a calibration curve of absorbance versus Inhibin concentration. The inhibin B concentrations in the samples can then be calculated from this calibration curve.

WARNING AND PRECAUTIONS

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

Use good laboratory practices.[23]

Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment or prior certification.[24] Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

Caution

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

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 U (20x)	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 samples.

Observe the following recommendations for handling, processing and storing blood samples:[25]

- Collect all blood samples observing routine precautions for venipuncture.
- Allow serum samples to clot completely before centrifugation.
- Keep tubes stoppered at all times.
- Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
- Serum and plasma samples may be stored at 2-8°C, if the assay is to be performed within 48 hours. For longer storage keep frozen (< -18°C, 6 months maximum), after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.

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.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Avoid repeated freezing and thawing of samples.

Avoid assaying lipemic, icteric or hemolyzed samples.

MATERIALS PROVIDED

Reagents of the kit may be labelled with IVD symbol, this is due to manufacturer internal purposes only. Do not take the IVD symbol into account. This kit is for research use only - not for use in diagnostic procedures.

Antibody Coated Microtitration strips: One stripholder

Containing 96 polystyrene microtitration wells with mouse monoclonal anti-activin B antibody immobilized to the inside wall of each well.

Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

Antibody-Biotin Conjugate Concentrate: One 0.4 mL vial

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

Store at 2 to 8°C until expiration date.

Dilute 10–30 minutes prior to use in Inhibin B Gen II Biotin Conjugate Diluent.

Streptavidin-Enzyme Conjugate: One 13.0 mL bottle (ready-to-use)

Containing conjugated HRP in buffer with protein (fish) and <10% methanol.

Store at 2 to 8°C until expiration date.

Assay Buffer: One 8 mL bottle (ready-to-use)

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

Store at 2 to 8°C until expiration date.

Biotin Conjugate Diluent: One 13 mL bottle (ready-to-use)

Containing buffer with BSA, protein (bovine, mouse, goat), surfactant, and <0.5% ProClin 300.

Store at 2 to 8°C until expiration date.

TMB Chromogen Solution: One 11 mL bottle (ready-to-use)

Containing a solution of TMB in citrate buffer with hydrogen peroxide.

Store at 2 to 8°C until expiration date.

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

The bottle contains borate buffer with TWEEN.**

Dilute the solution before use.

Store at 2 to 8°C or room temperature (18-25°C) until expiration date.

Stopping Solution A: One 11 mL bottle (ready-to-use)

The bottle is containing 0.2 M sulfuric acid.

Store at 2 to 8°C or room temperature (18-25°C) until expiration date.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

- **Inhibin B Gen II Calibrators and Controls A81302**
- Microtitration plate reader capable of absorbance measurement at 450/405 nm and preferentially capable of dual wavelength (reference filter) at 600 to 630 nm
- Deionized Water
- Precision pipette(s) to deliver 10–1000 µL
- Microtitration plate shaker capable of 600–800 orbital revolutions per minute (rpm)
- Microtitration plate washer
- Vortex mixer
- Absorbent materials for blotting the strips
- Graph paper for manual data reduction

Stop Solution (11 mL) may be ordered separately (cat # C24811).

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

Inhibin B Gen II Zero Calibrator (5 mL) may be ordered separately (cat # C35899).

PROCEDURE

Procedural notes

- A thorough understanding of this package insert is necessary for successful use of the Inhibin B Gen II ELISA.
- It is the responsibility of the customer to validate the assay for their use.
- Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- A calibration curve must be included with each assay.
- Bring all kit reagents to room temperature (18-25ºC) before use.
- Thoroughly mix the reagents before use by gentle inversion.
- Do not mix various lots of any kit component within an individual assay.
- Do not use any component beyond the expiration date shown on its label.
- 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 stopping solution into the wells in the same order and speed used to add the TMB chromogen solution.
- Avoid microbial contamination of reagents, especially of the conjugate and the assay buffer.
- Avoid contamination of the TMB chromogen solution with the conjugates.
- Use a clean disposable pipette tip for each reagent, calibrator, control or sample.
- 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.
- Use deionized water.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

Preparation of reagents

1. ***Wash Solution:*** Dilute one vial of wash concentrate with 950 mL of distilled water and mix thoroughly. 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.
2. ***Inhibin B Gen II Antibody-Biotin Conjugate:*** The Inhibin B Gen II Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part into 50 parts of Inhibin B Gen II Biotin Conjugate Diluent, according to the number of wells used. For an entire plate, pipet exactly 220 µL of the concentrate into 11 mL of the Inhibin B Biotin Conjugate Diluent.
   NOTE:The antibody-biotin conjugate concentrate should be freshly diluted 10–30 minutes prior to use.
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.

Assay procedure

Allow all samples and reagents to reach room temperature (18-25°C). Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipet 50 µL of the calibrators, controls and samples to the appropriate wells.
3. Add 50 µL of the Inhibin B Gen II Assay Buffer to each well using a precision pipette.
4. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for two hours at room temperature (18-25°C).
5. During the last 10-30 minutes of incubation, prepare the Inhibin B Gen II antibody-biotin conjugate solution by diluting the Inhibin B Gen II Biotin Conjugate Concentrate in Inhibin B Biotin Conjugate Diluent as described under the “Preparation of Reagents” section of this package insert.
6. Aspirate and wash each well five times with the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material.

NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, follow these steps to wash the plate manually:

(a) Completely aspirate the liquid from each well

(b) Dispense 350 µL of the wash solution into each well using a precision pipette

(c) Aspirate the liquid again

(d) Repeat steps (b) and (c) four times

7. Add 100 µL of the Inhibin B Gen II antibody-biotin conjugate solution to each well using a precision pipette.
8. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for one hour at room temperature (18-25°C).
9. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
10. Add 100 µL of the Inhibin B Gen II Streptavidin-Enzyme Conjugate solution to each well using a precision pipette.
11. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 30 minutes at room temperature (18-25°C).
12. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
13. Add 100 µL of the TMB chromogen solution to each well using a precision pipette.

Avoid exposure to direct sunlight.

14. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 8-12 minutes at room temperature (18-25°C).

NOTE: Be aware that the 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.

15. Add 100 µL of the stopping solution to each well using a precision pipette.
16. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm.

NOTE:

1) While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "Blank".

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

RESULTS

1. Calculate the mean absorbance for each calibrator, control or sample.
2. Subtract the mean absorbance of Calibrator 0 (blank) from the mean absorbance of calibrators 1-6, controls and samples. Plot the log of the mean blank-subtracted absorbance readings for calibrators 1-6 along the y-axis versus log of the inhibin B concentrations in pg/mL along the x-axis, using a cubic regression curve-fit. Alternatively, the data can be plotted log vs. log and a linear curve-fit can be used.
3. Determine the inhibin B concentrations of the controls and samples from the calibration curve by matching their mean absorbance readings with the corresponding inhibin B concentrations.
4. Any sample reading higher than the highest calibrator should be appropriately diluted using 0 pg/mL Calibrator 0 and reassayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. Multiply the value by a dilution factor, if required.
   NOTE: If the absorbance readings exceed the limitations of the plate reader, a second reading at 405 nm is needed (reference filter between 600 and 630 nm if available). In this case, proceed to construct a second calibration curve as above with the absorbance readings of all calibrators at 405 nm. The concentration of the off-scale samples at 450 nm is then read from the new calibration curve. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Standard curve

Calibrators	Conc. (pg/mL)	ABS	B/Bmax (%)
0	0	0.046 (blank)	-
1	10	0.035	1.10
2	31	0.117	3.68
3	101	0.436	13.7
4	259	0.941	29.6
5	560	1.929	60.7
6	1,177	3.179	100

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

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Inhibin B Gen II ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Inhibin B Gen II ELISA controls are provided with the Inhibin B Gen II Calibrator and Control kit.
- A full calibration curve, plus low and high level controls, should be included in each assay.
- The TMB chromogen solution should be colorless to very light yellow. Development of a blue color may indicate reagent contamination or instability.
- Quality control materials simulate the characteristics of samples and are essential for monitoring the system performance of immunochemical assays. Include QC or other commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results.
- 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
- In the US, contact the Beckman Coulter technical support at 1-800-854-3633; or by email at: immunoassay@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): 5.42 pg/mL

The LoD of the assay is 5.42 pg/mL, determined consistent with guidelines in CLSI document EP17-A2 [26] 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 [27]. 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 [28]).

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): 5.42 to approximately 1,000 pg/mL.

LIMITATIONS

- The reagents supplied in this kit are optimized to measure Inhibin B levels in serum and lithium heparin plasma.
- The possibility exists for interference by heterophile antibodies in the sample. Subjects 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 samples suspected of having these antibodies.

  Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in samples.[29,30]
- If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

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 [1,2]. 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 [3,4,5,6].

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 [7,8,9,10,11,12] and female [13,14,15,16,17,18,19,20,21] 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 [22]. 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. [31]. 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 [28]).

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)

APPENDIX - TRADEMARKS

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SOLN STOP — Stop Solution/ Solution d'arret / Stopp-Lösung/ Soluzione di arresto/ Solución de Parada / Solução de interrupção / Stopopløsning / Pysäytysliuos/ Διάλυμα αναστολής / Leállító oldat / Roztwór Stop / Stop roztok / Durdurma Çözeltisi / Стоп-раствор / „Stop" otopina / Стоп разтвор / Soluție inhibitoare / Rastvor za zaustavljanje / Stop-oplossing

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 / Soluție 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 / Biotiinikonjugaatti, konsentraatti / Συμπύκνωμα συζεύγματος βιοτίνης / Biotin konjugátum koncentrátum / Koncentrat koniugatu biotyny / Koncentrát konjugátu biotinu / Biotin Konjugat Konsantresi / Конюгат 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

SOLN TMB — TMB Chromogen Solution / Solution chromogène TMB / TMB-Chromgenlösung / Soluzione cromogena TMB / Solución de cromógeno TMB / Solução de cromogénio TMB / TMB-kromogenopløsning / TMB kromogen lösning / TMB-kromogeeniliuos / Διάλυμα χρωμογόνου TMB / TMB kromogén oldat / Roztwór chromogenu TMB / Roztok chromogenu TMB / TMB Kromojen Çözeltisi / Раствор хромогена TMB / TMB otopina kromogena / TMB хромогенен разтвор / Soluție cromogenă TMB / Rastvor TMB hromogena / TMB chromogeenoplossing

CONJ DIL — Conjugate Diluent/ Diluant pour le conjugué / Konjugatverdünnungsmittel / diluente del coniugato / Diluyente conjugado / Diluente de conjugado / Konjugatfortynder/ Konjugaatin diluentti / Αραιωτικό συζεύγματος / Konjugált díluens / Rozcieńczalnik koniugatu / Ředicí roztok konjugovaný / Konjugat dilüenti / Разбавитель для конъюгата / Diluent za konjugate / Конюгиращ буфер / Diluant pentru conjugat / Rastvor konjugata / Conjugaatverdunner

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 / Soluție 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

IFU — Instructions for Use / Mode d'emploi / Gebrauchsanweisung / Istruzioni per l'uso / Instrucciones de uso / Instruções de uso / Brugervejledningen / Bruksanvisning / Käyttöohjeet / Οι Οδηγίες χρήσης / Használati utasítás / Instrukcja użycia / Návod k použití / Kullanma Talimatı / Инструкции по применению / Upute za uporabu / Инструкциите за употреба / Instrucțiunile de utilizare / Uputstvo za upotrebu / Gebruiksaanwijzing

Biohazard / Risque biologique / Biogefährdung / Rischio biologico / Riesgo biológico / Risco biológico / Biologisk fare / Biologisk fara / Tartuntavaaraa / Βιολογικός κίνδυνος / Veszélyes biológiai anyag / Zagrożenie biologiczne / Biologické riziko / Biyolojik tehlike / Биологическая опасность / Biološka opasnost / Биологична опасност / Pericol biologic / Biogevaar

Symbols Key

REF	Product Reference / Référence du produit / Produktreferenz / Riferimento prodotto / Número de referencia del producto / Referência do produto / Produktreferens / Κωδικός αναφοράς προϊόντος / 产品参考 / Gaminio nuoroda / Termékszám / Dane referencyjne produktu / Reference k produktu / Referenčné označenie výrobku / 제품 참조 자료 / Ürün Referansı / Ссылка на продукт / Референция за продукт / 產品參考
IVD	In Vitro Diagnostic / Diagnostic in vitro / In-vitro-Diagnostikum / Diagnostica in vitro / Para diagnóstico in vitro / Diagnóstico in vitro / InVitro-diagnostik / Για διάγνωση in vitro / 体外诊断 / In vitro diagnostika / In vitro diagnosztikai felhasználásra / Diagnostyka in vitro / Diagnostika in vitro / 체외 진단 / In Vitro Diagnostik / Диагностика in vitro / За ин витро диагностика / 體外診斷
CONTENTS	Contents / Contenu / Inhalt / Contenuto / Contenido / Conteúdo / Περιεχόμενο / 组成 / Rinkinio sudėtis / Tartalom / Zawartość / Obsah / Obsah / 내용물 / İçindekiler / Содержание / Съдържание / 目錄
	Manufactured by / Fabriqué par / Hergestellt von / Prodotto da / Fabricado por / Tillverkas av / Κατασκευαστής / 制造商 / Gamintojas / Gyártó: / Producent / Výrobce / Výrobca / 제조 / Üretici / Изготовлено / Произведено от / 製造商
Σ	Contains sufficient for <n> tests / Contenu suffisant pour "n" tests / Inhalt ausreichend für <n> Prüfungen / Contenuto sufficiente per "n" saggi / Contenido suficiente para <n> ensayos / Conteúdo suficiente para "n" ensaios / Räcker till "n" antal tester / Περιεχόμενο επαρκές για "ν" εξετάσεις / 含量足够 <n> 次测试 / Turinio pakanka < n > tyrim / <n> teszthez elegendő mennyiséget tartalmaz / Zawartość wystarcza na <n> testów / Lze použít pro <n> testů / Obsah vystačí na < n > testov / <n> 테스트에 대해 충분한 양 포함 / <n> sayıda test için yeterlidir / Содержит достаточно для количества тестов: <n> / Съдържа достатъчно за <n> теста / 內容物足夠執行 <n> 次測試
CE	CE Mark / Marquage CE / CE-Kennzeichnung / Marchio CE / Marcado CE / Marcação CE / CE-märkning / Σήμανση CE / CE 标志 / CE ženklas / CE jelzés / Znak CE / Značka CE / Označenie CE / CE 표시 / CE İşareti / Маркировка CE / CE маркировка / CE 標識
SDS	Safety Data Sheet / Fiche technique santé-sécurité / Sicherheitsdaten-blatt / Scheda dati di sicurezza / Hoja de datos de seguridad / Ficha de Dados de Segurança / Säkerhetsdatablad / Φύλλο Δεδομένων Ασφάλειας / 安全数据单 / Saugos duomenų lapas / Biztonsági adatlap / Karta Charakterystyki Bezpieczeństwa / Bezpečnostní list / Bezpečnostný list / 안전보건자료 / Güvenlik Bilgi Formu / Паспорт безопасности / Информационен Лист За Безопасност / 安全性資料表
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	Caution / Précaution / Achtung / Attenzione / Precaución / Atenção / Försiktighet / Προσοχή / 注意事项 / Įspėjimas / Figyelem / Uwaga / Upozornění / Upozornenie / 주의 / Dikkat / Внимание / 注意
	Expiration Date / Date D'expiration / Verfallsdatum, Verw. bis: / Data Di Scadenza / Fecha De Caducidad / Data de validade / Utgångsdatum / Ημερομηνία λήξης / 失效日期 / Galiojimo data / Lejárati idő / Data ważności / Datum exspirace / Dátum exspirácie / 만료 날짜 / Son Kullanma Tarihi / Срок годности / Срок на годност / 到期日
LOT	Lot Number / Numéro de lot / Chargennummer / Numero di lotto / Lote número / Número de lote / Satsnummer / Αριθ. παρτίδας / 批次号 / partijos numeris / Tételszám / Numer serii / Číslo šarže / 로트 번호 / Lot Numarası / Номер партии / Номер на партида / 批號
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