Instructions for Use

Inhibin B ELISA

Enzyme immunoassay for the quantitative determination of Inhibin B in human serum.

REF **DS52021**

Σ **96**

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

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Inhibin B Gen II ELISA

CAUTION

Not for sale in U.S.A.

INTENDED USE

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. This assay is intended for *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

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 ß), 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 ß-subunit, and lacking inhibin bioactivity, are also present.[3,4,5,6] Inhibin B consists of an α-subunit and a ßB-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[22] that specifically recognize only the functional dimeric inhibin B molecule and does not measure the free α-subunit forms present in biological fluids. 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 OF THE TEST

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 concentrations in the samples can then be calculated from this calibration curve.

MATERIALS SUPPLIED

Ab PLATE **Inhibin B Gen II Antibody Coated Microtitration strips:**
A62384

- One stripholder, containing 96 polystyrene microtitration wells with 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.

BIO CONJ CONC **Inhibin B Gen II Antibody-Biotin Conjugate Concentrate:**
A69448

- Dilute 10–30 minutes prior to use in Inhibin B Gen II Biotin Conjugate Diluent.
- One vial, 0.4 mL, containing a solution of anti-Inhibin α–subunit antibody labeled biotin in a protein-based buffer with < 0.05% ProClin* 300.
- Store at 2 to 8°C until expiration date.

STREP CONJ RTU **Streptavidin-Enzyme Conjugate:**
A69450

- Provided ready-to-use.
- One bottle, 13 mL, containing conjugated HRP in a protein-based buffer and < 10% methanol.
- Store at 2 to 8°C until expiration date.

ASSAY BUFFER **Inhibin B Gen II Assay Buffer:**
A69447

- One bottle, 8 mL, containing a protein-based bovine serum albumin (BSA) buffer with < 0.25% ProClin 300.
- Store at 2 to 8°C until expiration date.

CONJ DIL **Inhibin B Gen II Biotin Conjugate Diluent:**
A69449

- One bottle, 13 mL, containing a protein based buffer with < 0.25% ProClin 300.
- Store at 2 to 8°C until expiration date.

TMB SOLN **TMB Chromogen Solution:**
DSL-10-9755

- One bottle, 11 mL, containing a solution of TMB in citrate buffer with hydrogen peroxide.
- Store at 2 to 8°C until expiration date.

WASHCONC B **Wash Concentrate B:**
DSL-10-9730

- One bottle, 60 mL, containing buffered saline with a nonionic detergent.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.
- Dilute 25-fold with deionized water prior to use.

STOP SOLN A **Stopping Solution A:**
DSL-10-9780

- One bottle, 11 mL, containing 0.2 M sulfuric acid.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. **Inhibin B Gen II Calibrators and Controls A81304.**
2. Microtitration plate reader capable of absorbance measurement at 450/405 nm and preferentially capable of dual wavelength (reference filter) at 630 nm
3. Deionized water
4. Precision pipette to deliver 10–1000 µL
5. Microtitration plate shaker capable of 600–800 orbital revolutions per minute (rpm)
6. Microtitration plate washer
7. Vortex mixer
8. Absorbent materials for blotting the strips
9. Graph paper for manual data reduction

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use.**
- Practice 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. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and samples as if capable of transmitting infectious disease.[24]
- Xi: Irritant: < 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- F: Highly Flammable: < 10% Methanol.

R 11: Highly flammable.
R 20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
R 68: Possible risk of irreversible effects.
S 16: Keep away from sources of ignition - No smoking.
S 36/37: Wear suitable protective clothing and gloves.
S 45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S 7: Keep container tightly closed.

Xn: Harmful:

- The Materials Safety Data Sheet (MSDS) is available upon request.

SAMPLE COLLECTION AND PREPARATION

- Serum and lithium heparin plasma are the recommended samples.
- Observe the following recommendations for handling, processing and storing blood samples:[25]
  a.) Collect all blood samples observing routine precautions for venipuncture.
  b.) Allow serum samples to clot completely before centrifugation.
  c.) Keep tubes stoppered at all times.
  d.) Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
  e.) Store samples tightly stoppered at 2 to 8°C for no longer than 48 hours.
  f.) If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C.
- Use the following guidelines when preparing samples:
  a.) Ensure residual fibrin and cellular matter have been removed prior to analysis.
  b.) 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 or hemolyzed samples.

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 (~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.

TEST PROCEDURE

Preparation of Reagents

1. ***Wash Solution:*** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (~25°C) when stored in a tightly sealed bottle.
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 (~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 (~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 to wash the plate manually:
   (a) Completely aspirate the liquid from each well
   (b) Dispense 0.35 mL 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 60 minutes at room temperature (~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 (~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 (~25°C).
    NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please 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. Plot the log of the mean absorbance 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. 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 A 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.

LIMITATIONS

- The reagents supplied in this kit are optimized to measure Inhibin B levels in serum and Li-Hep plasma.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Samples from individuals which 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. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in samples.[26,27]
- If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.
- The Inhibin B Gen II ELISA results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

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 printed on the control vial labels.
- A full calibration curve, low and high level controls, should be included in each assay.
- The TMB chromogen solution should be colorless. Development of a blue color may indicate reagent contamination or instability.
- Quality control materials simulate the characteristics of patient 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.

EXPECTED VALUES

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Serum samples were procured either from vendors or were aliquots stored in freezers from our previous studies. These samples were analyzed using 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	MEDIAN AGE (yrs)	MEDIAN IN (pg/mL)	2.5–97.5TH PERCENTILE IN (pg/mL)
Random Males (N=235)	35	166	25-325
Random Females (N=95)	30	47	ND-341
Females 3rd day of cycle (N=106)	NA	75	ND-273
Post Menopausal Females (N=20)*	74	ND	ND-4
Boys (N=15)**	11	93	4-352
Girls (N=15)**	11	18	ND-83

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

TYPICAL CALIBRATION CURVE

WELL NO.	WELL CONTENTS	MEAN ABSORBANCE	CONC. (pg/mL)
	CALIBRATORS		
A1, A2	A	(BLANK)	0
B1, B2	B	0.05	10
C1, C2	C	0.14	30
D1, D2	D	0.50	100
E1, E2	E	1.19	250
F1, F2	F	2.08	500
G1, G2	G	3.18	1000

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

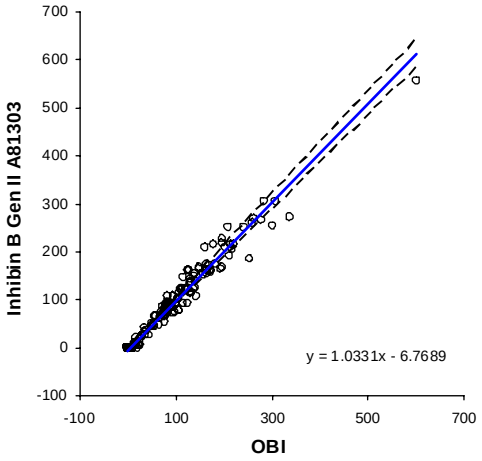
SPECIFIC PERFORMANCE CHARACTERISTICS

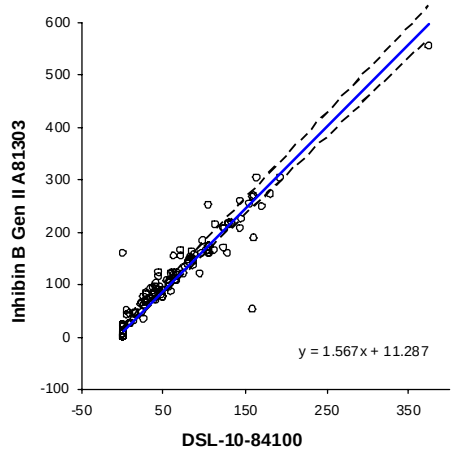
All analytical characteristics are stated in pg/mL.

Method Comparison

The Inhibin B Gen II (A81303) has been compared to two commercially available assays (Oxford Brooks 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 =1.03 (OBI) - 6.77 pg/mL
(r=0.99; P<0.0001)
Inhibin B Gen II = 1.57 (DSL-10-84100) + 11.29 pg/mL
(r=0.97; P<0.0001)





Dilution Recovery (Linearity)

Multiple dilutions of four samples containing various Inhibin B levels with Inhibin B Gen II Calibrator A (zero) resulted in the following data:[29]

SAMPLE	DILUTION FACTOR (1:X)	EXPECTED VALUE IN pg/mL	OBSERVED VALUE IN pg/mL	% RECOVERY
1		999.80	N/A	N/A
	2	499.90	492.40	98
	4	249.95	234.33	94
	8	124.97	117.66	94
	16	62.49	59.07	95
2		659.78	N/A	N/A
	2	329.89	319.12	97
	4	164.95	161.10	98
	8	82.47	86.54	105
	16	41.24	46.78	113
3		580.15	N/A	N/A
	2	290.07	256.85	89
	4	145.04	117.80	81
	8	72.52	66.31	91
	16	36.26	37.66	104
4		279.35	N/A	N/A
	2	139.67	135.08	97
	4	69.84	68.46	98
	8	34.92	36.08	103
	16	17.46	19.67	113

Spiking Recovery

Known amounts of Inhibin B were added to four 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	46.44	91.85	89.04	97
		133.13	128.47	97
		170.82	166.51	97
2	57.43	102.32	100.86	99
		143.12	132.37	92
		180.38	157.36	87
3	40.18	85.88	83.52	97
		127.43	124.15	97
		165.37	166.80	101
4	46.18	91.60	91.70	100
		132.89	158.48	119
		170.59	161.84	95

Imprecision:

Reproducibility of the Inhibin B Gen II assay was determined in a study using three serum samples (Q1, Q2, Q3) and two kit controls (C1, C2) with two lots of reagents. The study included a total of 40 assays, three replicates per assay.[28]

SAMPLE	MEAN CONC.	WITHIN RUN	BETWEEN RUN	TOTAL
	pg/mL	% CV	% CV	% CV
Q1	19.34	3.82	5.64	6.82
Q2	76.03	2.40	3.68	4.39
Q3	275.30	2.22	3.67	4.29
C1	99.88	2.67	4.70	5.40
C2	363.90	2.46	5.13	5.68

Analytical 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 reactants were non detectable.

Interference

When potential interferents (hemoglobin, triglycerides, bilirubin and human serum albumin), were added at least at two times their physiological concentration, Inhibin B concentrations were within ±10% of the control as represented in the following table.[30]

INTERFERENTS	ANALYTE CONC.	UNSPIKED SAMPLE (pg/mL)	SPIKED SAMPLE (pg/mL)	% DIFFERENCE TO REFERENCE
HEMOGLOBIN	2 mg/mL	102.66	93.91	-8.5
TRIGLYCERIDES	20 mg/mL	96.81	99.02	2.3
BILIRUBIN	0.6 mg/mL	91.40	97.06	6.2
HUMAN SERUM ALBUMIN	60 mg/mL	97.63	103.90	6.4

Limit of Detection (LoD):

The lowest amount of Inhibin B in a sample that can be detected with a 95% probability is 2.6 pg/mL. The value was determined by processing a complete seven point calibration curve, controls and seven serum samples in the range of zero to 105 pg/mL.[31] Two assay runs per day were performed over 10 days with all samples run in duplicate per run.

Limit of Quantitation (LoQ):

The estimated minimum dose achieved at 20% total imprecision is 4.8 pg/mL. The value was determined by processing a complete seven point calibration curve, controls and eight human serum samples with at least two samples that were less than the median of normal and minimum of three samples that were greater than the median of normal over 20 runs and 10 days in duplicates.[31]

REFERENCES

1 Muttukrishna, S and Ledger, W. ed. 2001 Inhibin, Activin and Follistatin in Human Reproductive Physiology. Imperial College Press, London, UK.

2 Welt, C, Sidis Y, Keutmann, H, Schneyer A. 2002 Activins, inhibins, and follistatins: from endocrinology to signaling. A paradigm for the new millennium. Exp. Biol. Med. 227 (9): 724-752.

3 Knight PG, Beard AJ, Wrathall HM, Castillo RJ. 1989 Evidence that the bovine ovary secretes large amounts of monomeric inhibin subunit and its isolation from bovine follicular fluid. J Mol Cell Endocrinol. 2:189-200.

4 Schneyer AL, Sluss PM, Whitcomb RW, Martin KA, Sprengel R, Crowley WF. 1991 Precursors of inhibin modulate FSH receptor binding and biological activity. Endocrinology. 129:1987-1999.

5 Robertson DM, Sullivan J, Watson M, Cahir N. 1995 inhibin forms in human plasma. J Endocrinol. 144:261-269.

6 Robertson D, Burger HG, Sullivan J, Cahir N, Groome N, Poncelet E, Franchimont P, Woodruff T, Mather JP. Biological and Immunological Characterization of inhibin Forms in Human Plasma. J Clin Endocrinol & Metab. 81:669-676.

7 Groome NP, Evans LW. 2000 Does measurement of inhibin have a clinical role? Ann Clin Biochem. 3:419-431.

8 Burger HG, Robertson DM. 1997 Editorial: inhibin in the male - progress at last. Endocrinol 138:1361-1362.

9 Klingmuller D, Haidl G. 1997 inhibin B in men with normal and impaired spermatogenesis. Hum Reprod. 12:2376-2378.

10 Pierik FH, Vreeburg JTM, Stijnen T, De Jong FH, Weber RFA. 1998 Serum inhibin B as a marker of spermatogenesis. J Clin Endocrinol Metab. 83:3110-3114.

11 Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, et al. 1996 Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab. 81:3341-3345.

12 Hayes FJ, Pitteloud N, DeCruz S, Crowley Jr. WF, Boepple PA. 2001 Importance of Inhibin B in the regulation of FSH secretion in the human male. J Clin Endocrinol Metab. 86:5541-5546.

13 Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS. 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab. 81:1401-1405.

14 Lockwood GM, Muttukrishna S, Ledger WL. 1998 inhibins and activins in human ovulation, conception and pregnancy. Hum Reprod. 4:284-295.

15 Corrine KW, McNicholl DJ, Taylor AE, Hall JE. 1999 Female reproductive aging is marked by decreased secretion of dimeric Inhibin. J Clin Endocrinol. 84:105-111.

16 Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Bert CA. 1997 Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. Fertil Steril. 67:110-114.

17 Seifer DB, Scott RT, Bergh PA, Abrogast LK, Friedman CI, Mack CK, Danforth DR. 1999 Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. Fertil Steril. 72:63-65.

18 Dzik A, Lambert-Messerlian G, Izzo VM, Soares JB, Pinotti JA, Seifer DB. 2000 inhibin B response to EFORT is associated with the outcome of oocyte retrieval in the subsequent in vitro fertilization cycle. Fertil Steril. 74:1114-1117.

19 Danforth DR, Arbogast LK, Mroueh J, Kim MH, Kennard EA, Seifer DB, et al. 1998 Dimeric inhibin: a direct marker of ovarian aging. Fertil Steril. 70:119-123.

20 Santoro N, Adel T, Skurnick JH. 1999 Decreased inhibin tone and increased activin A secretion characterize reproductive aging in women. Fertil Steril. 71:658-662.

21 Tinkanen H, Bläuer, Laippala P, Tuohimaa P, Kujansuu E. 2001 Correlation between serum inhibin B and other indicators of the ovarian function. Euro J Obst Gyn Reprod Bio. 94:109-113.

22 Ludlow, H, Muttukrishna, S, Hyvonen, M, Groome, N. P. 2008. Development of a new antibody to the human inhibin/activin B subunit and its application to improved inhibin B ELISAs. J Immunological Methods. 329: 102-111.

23 Clinical Laboratory Safety; Approved Guideline Second Edition, GP-17A2.

24 HHS Publication, 4th ed., April 1999. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

25 Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.

26 Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

27 Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.

28 Approved Guideline – Evaluation of precision performance of clinical chemistry devices, EP5-A. 1999, National Committee for Clinical Laboratory Standards.

29 Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

30 Interference Testing in Clinical Chemistry: Approved Guideline - Second Edition.

31 Protocol for Determination of Limits of Detection and Limits of Quantitation; Clinical and Laboratory Standards Institute: Approved Guideline. EP17-A, Vol. 24 No. 34.

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Printed in U.S.A.
Made in U.S.A.
Revised October 2009

Beckman Coulter consumables are being shipped with English Instructions for Use (IFUs). Where required, IFUs translated into multiple languages are available on-line at www.beckmancoulter.com/ifu. You may also contact your local Beckman Coulter sales representative or technical support organization to obtain translated IFUs.

Les consommables pour Beckman Coulter sont livrés avec des instructions d'utilisation en anglais. Si vous le souhaitez, vous pouvez accéder aux instructions traduites en plusieurs langues à l'adresse Internet www.beckmancoulter.com/ifu. N'hésitez pas à contacter votre société d'assistance technique ou votre représentant Beckman Coulter local pour obtenir des instructions traduites.

Die Verbrauchsmaterialien von Beckman Coulter werden mit englischer Gebrauchsanweisung (IFU) geliefert. Falls erforderlich, sind übersetzte Gebrauchsanweisungen in mehreren Sprachen online unter www.beckmancoulter.com/ifu verfügbar. Wenden Sie sich gegebenenfalls an Ihren zuständigen Vertreter von Beckman Coulter oder den technischen Kundendienst, um übersetzte Gebrauchsanweisungen zu erhalten.

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Los consumibles para Beckman Coulter se entregan con las instrucciones de uso (IFU) en inglés. Si lo desea, puede encontrar las IFU traducidas a varios idiomas en línea en la siguiente dirección: www.beckmancoulter.com/ifu. También puede ponerse en contacto con el representante local de ventas de Beckman Coulter o con la empresa de asistencia técnica para obtener las IFU traducidas.

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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Symbols Version 3.5 / 2011-07-01