

Inhibin A ELISA
REF DSL-10-28100t
CAUTION

Not for sale in U.S.A.

INTENDED USE

The Inhibin A enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of dimeric inhibin A in human serum or plasma. It is intended strictly for *in vitro* use as an aid in the diagnosis and monitoring of various hormonal reproductive disorders. Down syndrome (Trisomy 21) screening in the 2nd trimester using combined biochemical and ultrasound markers may be assessed with appropriate algorithms that can be run on commercially available risk calculation software.^{1,2} It is strongly recommended to use validated (CE marked) software designated specifically for evaluating the risk of Trisomy 21, e.g. software alpha.* The Inhibin A kit is intended for use in combination with hAFP + hCG + uE3.

SUMMARY AND EXPLANATION

Inhibins are heterodimeric protein hormones secreted by granulosa cells of the ovary in the female and Sertoli cells of the testis in the male. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and also have local paracrine actions in the gonads.^{3,4} The fully processed form of the inhibin molecule has a molecular weight of approximately 32 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.^{5,6,7,8} Inhibin A consists of an α -subunit and a β_A -subunit. Measurements of inhibin A are shown to be useful in studying its role in the human reproductive physiology.^{9,10,11} Several published reports indicate the utility of inhibin A measurement as an endocrine marker for monitoring ovarian function.^{12,13,14,15,16,17,18,19} Until recently, it was not possible to distinguish between circulating functional dimeric inhibin and free α -subunit in the normal human menstrual cycle. However, by using the highly characterized pair of antibodies, the two-site sandwich ELISA is found to be able to specifically measure only the dimeric inhibin A.²⁰

PRINCIPLE OF THE TEST

The Inhibin A ELISA is an enzymatically amplified two-step "sandwich" assay. In the assay, standards, controls and samples are incubated in microtitration wells which have been coated with anti-inhibin β_A subunit antibody. After incubation and washing, anti-inhibin alpha subunit detection antibody labeled with horseradish peroxidase (HRP) is added to each well. After a second incubation and washing step, the substrate tetramethylbenzidine (TMB) is added to the wells. Lastly, an acidic stopping solution is added. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm and between 600 and 630 nm. The absorbance measured is directly proportional to the concentration of inhibin A in the samples. A set of Inhibin A standards is used to plot a standard curve of absorbance versus inhibin A concentration. The inhibin A concentrations in the samples can then be calculated from this standard curve.

MATERIALS SUPPLIED

The 1-plate Inhibin A ELISA Kit contains sufficient reagents for 96 wells and the 4-plate Inhibin A ELISA Kit contains sufficient reagents for 384 wells. Each kit contains the following reagents:

AS PLATE Anti-Inhibin A Antibody Coated Microtitration strips:
 DSL-10-28110

- 1-Plate Kit: One strip-holder, containing 96 microtitration wells
- 4-Plate Kit: Four strip-holders, each containing 96 microtitration wells
- Polystyrene microtitration wells with anti-inhibin β_A 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.

STD A Inhibin A Standard A/Sample Diluent:
 DSL-10-28101

- 1-Plate Kit: One vial, 2.0 mL, labeled A
- 4-Plate Kit: Two vials, 2.0 mL, labeled A

- Zero (0) pg/mL recombinant dimeric inhibin A in bovine serum with < 0.5% ProClin** 300.
- Store unopened at 2 to 8°C until the expiration date.
- Stable at 2 to 8°C for 28 days after initial use. For longer periods store in a -20°C freezer (-15°C to -24°C) until expiration date.

STD B-G Inhibin A Standard B-G:
 DSL-10-28102, DSL-10-28103, DSL-10-28104, DSL-10-28105, DSL-10-28106, DSL-10-28107

- 1-Plate Kit: Six vials, 1.0 mL, labeled B-G
- 4-Plate Kit: Two sets (twelve vials), 1.0 mL, labeled B-G
- Concentrations of approximately 10, 30, 100, 250, 500 and 1000 pg/mL recombinant dimeric inhibin A in bovine serum with < 0.5% ProClin 300.
- Refer to vial labels for exact concentrations.
- Store unopened at 2 to 8°C until the expiration date.
- Stable at 2 to 8°C for 28 days after initial use. For longer periods store in a -20°C freezer (-15°C to -24°C) until expiration date.
- Provided ready to use.

The measurand (analyte) in the Inhibin A standards is traceable to the World Health Organization First International Standard for Inhibin-A (code 91/624) containing recombinant 32 kDa human inhibin A (1 pg/mL or 0.037 IU/mL). The assigned values were established using representative samples from this lot of standard and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CONTROL I-II Inhibin A Controls:
 DSL-10-28151, DSL-10-28152

- 1-Plate Kit: Two vials, 1.0 mL, labeled I and II
- 4-Plate Kit: Two sets (four vials), 1.0 mL, labeled I and II
- Low and high concentrations of recombinant dimeric inhibin A in bovine serum with < 0.5% ProClin 300.
- Refer to vial labels for exact control ranges.
- Store unopened at 2 to 8°C until the expiration date.
- Stable at 2 to 8°C for 28 days after initial use. For longer periods store in a -20°C freezer (-15°C to -24°C) until expiration date.
- Provided ready to use.

SAMPLE BUF A Inhibin A Sample Buffer A:
 DSL-10-28150-1

- 1-Plate Kit: One bottle, 10.0 mL
- 4-Plate Kit: Four bottles, 10.0 mL
- Buffer with bovine serum albumin (BSA), animal serum (goat, mouse), surfactant, and sodium azide.
- Store at 2 to 8°C until expiration date.

SAMPLE BUF B Inhibin A Sample Buffer B:
 DSL-10-28155-1 or DSL-10-28155-4

- 1-Plate Kit: One bottle, 10.0 mL
- 4-Plate Kit: One bottle, 30.0 mL
- Buffer with < 20% urea hydrogen peroxide, < 0.5% ProClin 300, and sodium azide.
- Store at 2 to 8°C until expiration date.

CONJ CONC 50X Inhibin A Antibody-Enzyme Conjugate Concentrate:
 DSL-10-28120

- 1-Plate Kit: One vial, 0.6 mL
- 4-Plate Kit: Four vials, 0.6 mL
- Mouse monoclonal anti-inhibin α -subunit antibody-HRP conjugate, MOPSO, BSA and < 1.0% ProClin 300.
- Store at 2 to 8°C until the expiration date.
- Dilute prior to use in Conjugate Diluent.

TMB SOLN TMB Chromogen Solution:
 DSL-10-9755-1 or DSL-10-9755-4

- 1-Plate Kit: One bottle, 15.0 mL
- 4-Plate Kit: One bottle, 50.0 mL
- Tetramethylbenzidine (TMB) in citrate buffer with hydrogen peroxide.
- Store at 2 to 8°C until expiration date.

Inhibin-A Sample Buffer B



DANGER

H314 Causes severe skin burns and eye damage.

H317 May cause an allergic skin reaction.

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.

P333+P313 If skin irritation or rash occurs: Get medical advice/attention.

P362+P364 Take off contaminated clothing and wash it before use.

Urea Hydrogen Peroxide 10 - 20%

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%



Inhibin-A Conjugate Diluent



WARNING

H316 Causes mild skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

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.

Tris(hydroxymethyl)-aminomethane 1 - 5%

octylphenoxypoly(ethoxyethanol) 1.5 - 2.5%

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%

Stopping Solution A



DANGER

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves, protective clothing and eye/face protection.

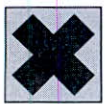
P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting. P303+P361+P353 IF ON SKIN (or hair): Rinse skin with water.

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%

Xi: Irritant: < 1.0% ProClin 300, < 2% MOPSO.



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.

C: Corrosive: < 20% Urea Hydrogen Peroxide.

R 34: Causes burns.

S 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 27: Take off immediately all contaminated clothing.

S 28: After contact with skin, wash immediately with plenty of soap and water.

S 36: Wear suitable protective clothing.

S 37: Wear suitable gloves.

S 39: Wear eye/face protection.



- **SDS** The Safety Data Sheet (SDS) is available upon request or at techdocs.beckmancoulter.com

SAMPLE COLLECTION AND PREPARATION

- Serum and plasma are the recommended samples.
- Observe the following recommendations for handling, processing and storing blood samples:²³
 - a.) Collect all blood samples observing routine precautions for venipuncture.
 - b.) Keep tubes stoppered at all times.
 - c.) Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
 - d.) Store undiluted or diluted samples tightly stoppered at 2 to 8°C for no longer than 24 hours.
 - e.) If the assay will not be completed within 24 hours, or for shipment of samples, freeze at -20°C or colder for up to 30 days.
- 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 A 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 standard curve must be included with each assay.
- Bring all kit reagents to room temperature (~25°C) 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.
- Handle all reagents carefully to avoid introducing microbial contaminants which can damage the reagents, especially the Conjugate Diluent and Sample Buffer A.
- Avoid contamination of the TMB chromogen solution with the conjugates.
- Use a clean disposable pipette tip for each reagent, standard, 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.

proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

TYPICAL STANDARD CURVE

WELL NO.	WELL CONTENTS	MEAN ABSORBANCE	CONC. (pg/mL)
STANDARDS			
A1, A2	A	0.022 (Blank)	0.0
B1, B2	B	0.032	10
C1, C2	C	0.083	30
D1, D2	D	0.280	100
E1, E2	E	0.664	250
F1, F2	F	1.293	500
G1, G2	G	2.530	1000

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

EXPECTED VALUES

Each laboratory should establish its own reference ranges to assure proper representation of specific populations. The following values presented were obtained with the Inhibin A ELISA using serum samples from apparently normal healthy adults. All values are reported in pg/mL. For normally cycling females, numbers beneath each phase represent days before or after LH surge.

POPULATION	N	MEAN (pg/mL)	MEDIAN (pg/mL)	95% CONFIDENCE RANGE (pg/mL)
Normally Cycling Females				
Early Follicular Phase (-14 to -10)	136	13.48 ± 0.93	10.54	†5.46 - 28.16
Mid Follicular (-9 to -4)	228	18.63 ± 0.59	17.06	†7.87 - 34.54
Late Follicular (-3 to -1)	130	56.10 ± 2.30	52.19	19.49 - 102.28
Mid Cycle (Day 0)	42	98.87 ± 30.99	98.23	49.92 - 155.48
Early Luteal (1 to 3)	115	71.59 ± 3.39	67.24	35.93 - 132.68
Mid Luteal (4 to 11)	268	75.22 ± 2.63	72.51	†3.15 - 159.55
Late Luteal (12 to 14)	82	27.87 ± 3.20	17.44	†7.28 - 89.95
IVF Peak levels	43	792.37 ± 70.63	705.91	354.2 - 1690.0
PCOS - Ovulatory	26	†9.32 ± 2.61		†5.65 - 15.99
Postmenopausal	23	†1.15 ± 0.24		†< 1 - 3.88
Normal Males	40	†1.33 ± 0.42		†< 1 - 3.58

†Inhibin A values below Standard B are extrapolated.

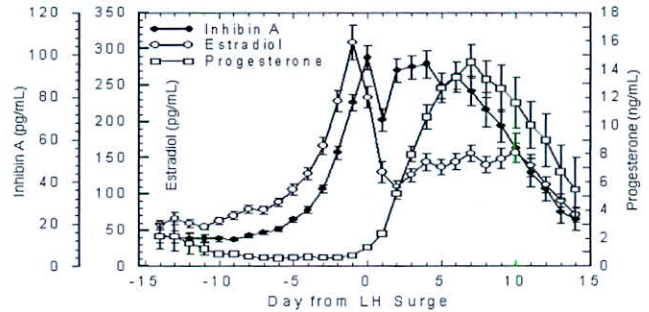
The values represented in the following table are maternal serum inhibin A in the second trimester.

COMPLETED WEEK	NUMBER OF SAMPLES	MEDIAN INHIBIN A (pg/mL)	LOG ₁₀ SD
15	30	157.55	0.27
16	124	153.29	0.27
17	74	151.20	0.27
18	45	155.14	0.27
19	20	165.18	0.27
20	16	185.76	0.27

Table adapted from values provided by a US screening center (2005).

Figure 1: Physiological Profiles of Inhibin A, Estradiol and Progesterone During the Normal Ovulatory Cycle

The mean ± SE concentrations of inhibin A, estradiol, and progesterone in 35 subjects (9 subjects with two cycles each, total 44 normal cycles) are shown, aligned relative to the day of the mid cycle LH peak. The inhibin A levels remained low (13.6 pg/mL) during the early follicular phase and rose in the late follicular phase to reach a peak concentration (98.9 pg/mL) on the day of the LH peak. After the LH peak, inhibin A levels fell briefly, then rose again during early to mid luteal phase, and fell again in concert with the drop in progesterone during the late luteal phase (28.3 pg/mL). Estradiol and inhibin A were highly correlated during the follicular phase (day -14 to -2) as evident by the following linear regression data:

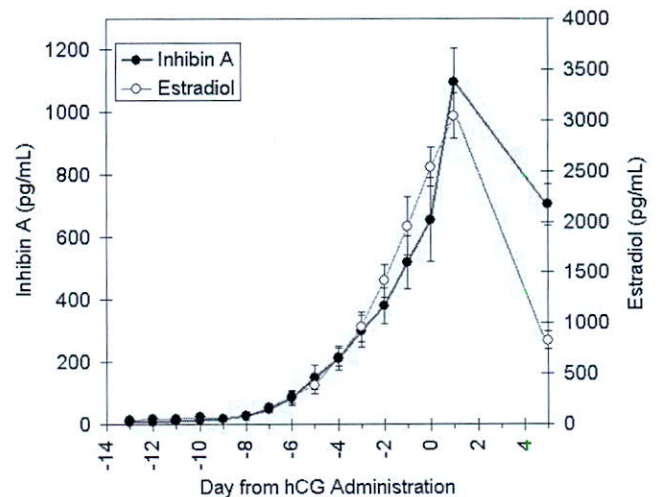


Inhibin A = 0.18 [Estradiol] + 3.3 Inhibin A range†: 3.0 - 82.8 pg/mL
n = 433 Estradiol range: 5.0 - 359 pg/mL
r = 0.80 95% Confidence interval for slope = 0.17 - 0.19
p < 0.001 95% Confidence interval for intercept = 1.8 - 4.8

†Inhibin A values below Standard B are extrapolated.

Figure 2: Inhibin A and Estradiol Profiles in Ovulation Induction Cycles

The mean ± SE concentrations of inhibin A and estradiol in 20 subjects during ovarian stimulation for IVF are shown, aligned to the day of hCG treatment.



Linearity

Five serum samples were diluted with 0 pg/mL Inhibin A Standard and assayed.

SAMPLE	DILUTION (% OF NEAT SAMPLE)	EXPECTED (pg/mL)	OBSERVED (pg/mL)	RECOVERY (%)
I	Neat	---	789.9	---
	50%	394.9	415.5	105
	25%	197.5	199.7	101
	12.5%	98.7	98.6	100
	6.25%	49.4	47.0	95
	MEAN % RECOVERY			100
II	Neat	---	1028.2 [†]	---
	50%	514.1	512.0	100
	25%	257.0	259.7	101
	12.5%	128.5	121.2	94
	6.25%	64.3	59.4	92
	MEAN % RECOVERY			97
III	Neat	---	891.1	---
	50%	445.5	476.1	107
	25%	222.8	232.3	104
	12.5%	111.4	126.1	113
	6.25%	55.7	58.4	105
	MEAN % RECOVERY			107
IV	Neat	---	897.5	---
	50%	448.7	461.8	103
	25%	224.4	240.0	107
	12.5%	112.2	108.5	97
	6.25%	56.1	53.2	95
	MEAN % RECOVERY			100
V	Neat	---	1090.4 [†]	---
	50%	545.2	626.6	115
	25%	272.6	294.0	108
	12.5%	136.3	132.6	97
	6.25%	68.2	69.2	101
	MEAN % RECOVERY			105
OVERALL MEAN % RECOVERY				102

[†]Inhibin A values above Standard G are extrapolated.

Specificity

The Inhibin A ELISA assay is highly specific for inhibin A. The following substances did not exhibit cross-reactivity in the Inhibin A ELISA.

HORMONE	CONCENTRATION TESTED
Inhibin B	1 µg/mL
Activin-A	1 µg/mL
Activin-B	1 µg/mL
Pro-Alpha C	0.27 µg/mL

Interference

The following substances in the amounts listed did not significantly interfere with the measurement of inhibin A in the Inhibin A ELISA.

SUBSTANCE	CONCENTRATION TESTED	SUBSTANCE	CONCENTRATION TESTED
Bilirubin (conjugated)	5 mg/dL	Aminophylline	100 µg/mL
Triglycerides	500 mg/dL	Acetaminophen	100 µg/mL
Hemoglobin	100 mg/dL	Caffeine	100 µg/mL
Follistatin	1 µg/mL	Ibuprofen	100 µg/mL
Alpha 2 Macroglobulin	10 µg/mL	Indomethacin	100 µg/mL
HSA	6 g/dL	Acetylsalicylic Acid	100 µg/mL
Catalase	1 units/mL	Ascorbic Acid	100 µg/mL

Comparison of Inhibin A and Estradiol

Two hundred forty-six samples were collected from subjects scheduled for IVF at two different centers. Inhibin A was measured by ELISA and estradiol was measured by two separate commercially available kits. Regression analysis yielded the following:

$$\text{Inhibin A} = 0.247 [\text{Estradiol}] + 67.6$$

n = 246
r = 0.85
p < 0.0001

Inhibin A range[†] = 2.2 - 1395 pg/mL
Estradiol range = 10 - 5960 pg/mL
95% Confidence interval for slope = 0.227 - 0.267
95% Confidence interval for intercept = 44.1 - 91.2

[†]Inhibin A values below Standard B are extrapolated.

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