Inhibin A

diasino

Inhibin A (ELISA)

REF: DS197700 Σ **96**

Intended use
The DiaSino Inhibin A assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of dimeric inhibin A in human serum. This assay can be used as an aid in the diagnosis and monitoring of various hormonal reproductive disorders. For professional use only.

Summary
Inhibins are dimeric glycoproteins composed of an α-subunit and either a βA (inhibin A) or a βB-subunit (inhibin B). The inhibin α-subunit has N-linked glycosylation sites and the glycosylation is essential for inhibin bioactivity (FSH suppressing activity). Inhibins are mainly produced in gonads and provide negative regulation of FSH secretion, but also have paracrine/autocrine actions in gonads and adrenal gland. As they sharing the β-subunits with activins, it is now recognized that actions of inhibins are associated with their antagonism of activin signaling.
In both females and males, inhibin inhibits FSH production. Inhibin does not inhibit the secretion of GnRH from the hypothalamus.[1,2] However, the overall mechanism differs between the sexes:
In females, inhibin is produced in the gonads, pituitary gland, placenta, corpus luteum and other organs. FSH stimulates the secretion of inhibin from the granulosa cells of the ovarian follicles in the ovaries. In turn, inhibin suppresses FSH. Inhibin B reaches a peak in the early- to mid-follicular phase, and a second peak at ovulation. Inhibin A reaches its peak in the mid-luteal phase. Inhibin secretion is diminished by GnRH, and enhanced by insulin-like growth factor-1 (IGF-1).
In males, it is secreted from the Sertoli cells,[3] located in the seminiferous tubules inside the testes. Androgens stimulate inhibin production; this protein may also help to locally regulate spermatogenesis.[4]

Test principle
Two-step sandwich principle. Total duration of assay: **140 minutes.**
The Inhibin A ELISA is a two-step sandwich assay. In the assay, calibrators, controls and samples are incubated in wells, which have been coated with anti-inhibin βA subunit antibody. After incubation and washing, anti-inhibin α 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, stop 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 calibrators 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.

Reagents
Materials provided
- **Inhibin A Coated Microplate - symbol** INHA PLATE 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal βA subunit antibody.
- **Inhibin A Enzyme Conjugate - symbol** INHA CONJ 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal anti-inhibin α subunit antibody in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
- **Inhibin A Calibrators - symbols** INHA CAL A-F 6 vials, 1.5 mL each, ***ready to use,*** Concentrations: 0(**A**), 20(**B**), 50(**C**), 200(**D**), 600(**E**) and 1200(**F**) pg/mL.
- **Inhibin A Sample Buffer A - symbol** INHA BUFFER A 1 vial, 3.0 mL. Contains 0.2% ProClin 300 preservative
- **Inhibin A Sample Buffer B - symbol** INHA BUFFER B 1 vial, 3.0 mL. Contains Bovine serum albumin (BSA), animal serum (goat, mouse), and surfactant.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 2 pieces.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 µL with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05%-0.2% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8℃.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino Inhibin A ELISA has been standardized against the World Health Organization International preparation NIBSC code 91/624 version 3.0. Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)

IVD CE

Inhibin A

diasino

Inhibin A (ELISA)

Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure

Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.

1. Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
2. Add **100 µL** of calibrators, controls, and samples to the appropriate well.
3. Add **25 µL** of the **Sample Buffer A** to each well using a precision pipette.
4. Add **25 µL** of the **Sample Buffer B** to each well using a precision pipette.
5. Cover the plate with a plate lid, and **incubate** the wells, **shaking at 500–700 rpm** on an orbital microplate shaker, for **60 minutes at room temperature *(OD value shows best between 20-25℃)***.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add **100 µL** of **Conjugate** to each well.
9. Cover the plate with a plate lid, and **incubate** the wells, **shaking at 500–700 rpm** on an orbital microplate shaker, for **60 minutes at room temperature *(OD value shows best between 20-25℃).***
10. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
11. Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
12. Add **100 µL** of **substrate** to each well.
13. Cover and i**ncubate** at **room temperature (18-25℃)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
14. Add **50 µL** of **stop solution** to each well.
15. Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
16. Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation

- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in pg/mL for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Calibrators	Value (pg/mL)	Absorbance 450nm
A	0	0.025
B	20	0.117
C	50	0.376
D	200	0.771
E	600	1.204
F	1200	1.741

Limitations - interference

- The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or < 23 ng/mL.
- Criterion: Recovery within ± 10 % of initial value.
- Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
- 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 antigoat antibodies may be present.[7, 8]
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

4.96-1200 pg/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 4.96 pg/mL. Values below the detection limit are reported as < 4.96 pg/mL. Values above the measuring range are reported as > 1200 pg/mL.

Lower detection limit

4.96 pg/mL

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n=21).

Expected values

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

Population	n	Median	95% confidence range
Normally Cycling Females			
Early Follicular Phase (-14 to -10)	89	11.65	5.78-27.34
Mid Follicular (-9 to -4)	86	18.24	7.93-32.56
Late Follicular (-3 to -1)	87	57.66	21.79-104.76
Mid Cycle (Day 0)	35	102.34	51.23-161.13
Early Luteal (1 to 3)	84	65.21	37.89-129.08
Mid Luteal (4 to 11)	82	69.88	14.12-154.67
Late Luteal (12 to 14)	77	16.74	9.87-91.23
Postmenopausal	28	3.98	2.32-5.23
Normal Males	36	2.56	2.1-3.88

The values represented in the following table were obtained with the DiaSino Inhibin A ELISA using maternal serum samples in the second trimester.

Completed week	No. of samples	Median Inhibin A (pg/mL)
15	56	151.25
16	87	147.14
17	79	145.36
18	55	149.76
19	37	158.21
20	21	181.22

Specific performance data

Representative performance data are given below.
Results obtained in individual laboratories may differ.

Precision

Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human Serum 1	58.1	4.74	8.15	5.60	9.64
Human Serum 2	173.9	12.97	7.46	13.20	7.59
Human Serum 3	366.4	23.01	6.28	26.16	7.14
PC Universal 1	164.2	13.91	8.47	15.17	9.24
PC Universal 2	408.7	29.14	7.13	34.41	8.42

*Repeatability = within-run precision

Analytical 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.

IVD CE

Inhibin A

diasino

Inhibin A (ELISA)

Hormone	Concentration Tested
Inhibin B	1 µg/mL
Activin A	1 µg/mL
Activin B	1 µg/mL
Pro-Alpha C	0.29 µg/mL

Method comparison
A comparison of the DiaSino Inhibin A ELISA (y) with commercially available Inhibin A ELISA (x) using 89 pregnant female serum samples gave the following correlations. The sample concentrations were between approx. 0 and 559 pg/mL.
Linear regression
y = 0.8929x + 24.441
r = 0.9728

Functional sensitivity
5.08 pg/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %

References

1. Luisi S, Florio P, Reis FM, Petraglia F (2005). "Inhibins in female and male reproductive physiology: role in gametogenesis, conception, implantation and early pregnancy". Human Reproduction Update. 11 (2): 123–35. doi:10.1093/humupd/dmh057. PMID 15618291.
2. Le T, Bhushan V, Hofmann J (2012). First Aid for the USMLE Step 1. McGraw Hill. p. 534. ISBN 978-0-07-177636-3.
3. Skinner MK, McLachlan RI, Bremner WJ (October 1989). "Stimulation of Sertoli cell inhibin secretion by the testicular paracrine factor PModS". Molecular and Cellular Endocrinology. 66 (2): 239–49. doi:10.1016/0303-7207(89)90036-1. hdl:1773/4395. PMID 2515083. S2CID 21885326.
4. Meachem SJ, Nieschlag E, Simoni M (November 2001). "Inhibin B in male reproduction: pathophysiology and clinical relevance". European Journal of Endocrinology. 145 (5): 561–71. doi:10.1530/eje.0.1450561. PMID 11720872.

Symbols

IVD **In vitro diagnostic medical device**	8°C 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**	CE **European Conformity**	EC REP **Authorized representative in the European Community**

diasino
DiaSino Laboratories Co., Ltd
No.68 Jingnansi Road National Eco & Tech Development Area
Zhengzhou 450000, P.R. China. info@diasino.com

EC REP **CMC Medical Devices & Drugs S.L**
C/Horacio Lengo Nº 18, CP 29006 Málaga, Spain. Tel: +34 9512 14054

IVD CE