**INTENDED USE**

The Inhibin A enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of dimeric inhibin A in human serum. It is intended for in vitro diagnostic 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 risk calculation software. It is strongly recommended to use validated software (CE marked) designated specifically for evaluating the risk of Trisomy 21 using Inhibin A in combination with hAFP, hCG, and uE3 measurements.

**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. The fully processed form of the inhibin molecule has a molecular weight of approximately 32 kD and consists of 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. Inhibin A consists of an α-subunit and β-subunit. Measurements of Inhibin A are shown to be useful in studying its role in human reproductive physiology. In uncomplicated pregnancy, the maternal serum level of inhibin A first decrease, and then remain relatively constant in the first and second trimesters of pregnancy. In the second trimester of pregnancy, inhibin A concentrations are, on average, doubled in pregnancies affected by Down syndrome. When screening is performed in the second trimester, upgrading concentrations in animal sera and a non-mercury preservative. Refer to calibration card for exact control concentrations. Store unopened at 2-8°C until the expiration date. Reconstitute control Levels I and II with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

**MATERIALS SUPPLIED**

- **CAL-123A** Inhibin A Calibrator A (Lyophilized)
  - One vial, labeled A, containing 0 pg/mL Inhibin A in animal sera and a non-mercury preservative. Store unopened at 2-8°C until the expiration date. Reconstitute calibrator A with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

- **CAL-123B - CAL-123F** Inhibin A Calibrators B - F (Lyophilized)
  - Five vials, labeled B-F, containing concentrations of approximately 10-1200 pg/mL Inhibin A in animal sera and a non-mercury preservative. Refer to calibration card for exact control concentrations. Store unopened at 2-8°C until the expiration date. Reconstitute calibrators B-F with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

**STANDARDIZATION NOTE**: The Inhibin A calibrators are traceable to the World Health Organization International preparation NIBSC code 91/624 version 3.0.

**PRINCIPLE OF THE TEST**

The Inhibin A ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Inhibin A antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Inhibin A antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. 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 reference filter. The absorbance measured is directly proportional to the concentration of Inhibin A in the samples and calibrators.

**MATERIALS SUPPLIED**

- **CTR-123-I & CTR-123-II** Inhibin A Controls I & II (Lyophilized)
  - Two vials, labeled Levels I and II containing low and high Inhibin A concentrations in animal sera and a non-mercury preservative. Refer to calibration card for exact control ranges. Store unopened at 2-8°C until the expiration date. Reconstitute control Levels I and II with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

- **PLT-123** Inhibin A Coated Microtitration Strips
  - One strip holder, containing 12 strips and 96 microtitration wells with Inhibin A antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

- **ASB-123A** Inhibin A Assay Buffer A
  - One bottle, 6 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

- **ASB-123B** Inhibin A Assay Buffer B
  - One bottle, 6 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

- **BCC-123** Inhibin A Biotin Conjugate Concentrate
  - One vial, 0.4 mL, containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute prior to use in Inhibin A Conjugate diluent. Store at 2-8°C until expiration date.
CND-123  Inhibin A Biotin Conjugate Diluent
One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-123  Inhibin A Streptavidin-Enzyme Conjugate—Ready-to-Use (RTU)
One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100  TMB Chromogen Solution
One bottle, 12 mL, containing a solution of Tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100  Stopping Solution
One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2-30°C until expiration date.

WSH-100  Wash Concentrate A
One bottle, 60 mL, containing phosphate buffer saline solution with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Microplate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 μL.
5. Vortex mixer.
6. Deionized water.

WARNINGS AND PRECAUTIONS
For in vitro-diagnostic use.
The following precautions should be observed:

a) Follow good laboratory practice.
b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
c) Handle and dispose of all reagents and materials in compliance with applicable regulations.
d) If external package is damaged, inspect the components inside for any other damage. Do not use if the components are damaged.

WARNING: Potential Biohazardous Material
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.

WARNING: Potential Chemical Hazard
Some reagents in this kit contain Pro-Clean 400 and Sodium azide as a preservative. Pro-Clean 400 and Sodium Azide in concentrated amounts are irritants to skin and mucous membranes. For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION
a) Serum is the recommended sample type.
b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer’s instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.
c) Samples may be stored at 4°C if assayed within 24 hours; otherwise, samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
d) Avoid assaying lipemic, hemolyzed or icteric samples.
e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.
f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

PROCEDURAL NOTES
1. A thorough understanding of this package insert is necessary for successful use of the Inhibin A ELISA assay. It is the user’s responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature (23 ± 2°C) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. 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.
5. 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 substrate solution into the wells. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

PREPARATION OF REAGENTS
1. Inhibin A Calibrators A-F and Inhibin A Controls I & II: Tap and reconstitute Inhibin A Calibrators A-F and Inhibin A Controls I & II each with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution.
2. Wash Solution: Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
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.
4. Inhibin A Antibody-Biotin Conjugate Solution: The Inhibin A Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Inhibin A Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220 μL of the Concentrate into each 1 mL of the diluent.
5. Inhibin A Assay Buffers Premix Solution: The Inhibin A Assay Buffer A (ASB-123A) and Inhibin A Assay Buffer B (ASB-123B) should be mixed by gentle inversion in equal volumes (1:1 ratio) according to the number of wells used. If an entire plate is to be used mix exactly 3 mL of the ASB-123 B in to 3 mL of the ASB-123 A. The pre-mixture solution is stable for use up to 4 hours. Discard the pre-mix solution after 4 hours.
ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

NOTE: All serum samples reading higher than the highest calibrator should be mixed and diluted in the 0 pg/mL reconstituted Calibrator A prior to assay.

1. Reconstitute Inhibin A Calibrators A-F and Inhibin A Controls I & II each with 1.0 mL deionized water. Solubilize for 10 minutes and mix well.
2. Label the microtiter strips to be used.
3. Pipette 50 μL of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add 50 μL of Inhibin A Assay Buffers Premix Solution (ASB-123A and ASB-123B in 1:1 ratio as described under the Preparation of the Reagents section of this package insert) to each well using a repeater pipette.
5. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 2.5 hour at room temperature (23 ± 2°C).
6. During the last 20-30 minutes of incubation, prepare the Inhibin A Antibody-Biotin Conjugate Solution by diluting the Inhibin A Biotin Conjugate Concentrate in Inhibin A Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
7. Aspirate and wash each strip 5 times with Washing Solution (350 μL per well) using an automatic microplate washer.
8. Add 100 μL of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 1 hour at room temperature (23 ± 2°C).
10. Aspirate and wash each strip 5 times with the Wash Solution (350 μL per well) using an automatic microplate washer.
11. Add 100 μL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 30 minutes at room temperature (23 ± 2°C).
13. Aspirate and wash each strip 5 times with the Wash Solution (350 μL per well) using an automatic microplate washer.
14. Add 100 μL of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 10-12 min at room temperature (23 ± 2°C).

NOTE: Visually monitor the color development to optimize the incubation time.
16. Add 100 μL of the stopping solution to each well using a repeater pipette. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.

NOTE: Zero calibrator should be programmed as “Blank” while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

RESULTS

NOTE: The results in this package insert were calculated by plotting the log optical density (OD) data on the y-axis and log Inhibin A concentration on X-axis using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.

1. Optimum results can be obtained at incubation temperature of 23 ± 2°C.
2. Calculate the mean OD for each calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Inhibin A concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the Inhibin A concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Inhibin A concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (CAL A) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Inhibin A levels in human serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples. The Inhibin A ELISA results should be interpreted with respect to the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate patient examination information.

QUALITY CONTROL

• Each laboratory should establish mean values and acceptable ranges to assure proper performance.
• Inhibin A ELISA controls or other commercial controls should fall within established confidence limits.
• The confidence limits for Inhibin A controls are printed on the Calibration card.
• A full calibration curve, low- and high-level controls, should be included in each assay.
• TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

<table>
<thead>
<tr>
<th>Well Number</th>
<th>Well Contents</th>
<th>Mean OD</th>
<th>Conc (pg/mL)</th>
</tr>
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<tbody>
<tr>
<td>A1, A2</td>
<td>Calibrators A</td>
<td>0.07</td>
<td>Blank</td>
</tr>
<tr>
<td>B1, B2</td>
<td>C</td>
<td>0.04</td>
<td>9.9</td>
</tr>
<tr>
<td>C1, C2</td>
<td>D</td>
<td>0.12</td>
<td>26.4</td>
</tr>
<tr>
<td>D1, D2</td>
<td>E</td>
<td>0.40</td>
<td>99.0</td>
</tr>
<tr>
<td>E1, E2</td>
<td>F</td>
<td>1.14</td>
<td>323.4</td>
</tr>
<tr>
<td>F1, F2</td>
<td></td>
<td>3.06</td>
<td>1188.0</td>
</tr>
</tbody>
</table>

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

ANALYTICAL CHARACTERISTICS

Analytical Sensitivity:
The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 42 replicates in 21 runs of calibrator A (0 pg/mL) and calibrator B (9.9 pg/mL) is 5.45 pg/mL.

Imprecision:
Reproducibility of the Inhibin A assay was determined in a study using Four serum pools. The study included a total of 21 assays, four replicates of each per assay (n=84). Representative data calculated are presented in the following table.
Refer to package insert included with the product for exact specifications.

The concentration of Inhibin A was determined before and after the addition of exogenous Inhibin A and the percent recovery was calculated.

**Linearity:**

Based on NCCLS EP-6-P, multiple dilutions of the four serum samples and Calibrator F containing various Inhibin A levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

**Analytical Specificity:**

This monoclonal antibody pair used in the assay detects Inhibin A. Other related molecules at the concentration in the table below did not show any significant cross-reaction. Specificity to other species has not been determined.

**Interference:**

When hemoglobin, triglycerides and biotin were added at a concentration greater than two folds of their physiological concentration to control sample, average Inhibin A concentration were within ±15% of the control as represented in the following table.

**Expected Value:**

Serum samples were analyzed using Ansh Inhibin A ELISA. The expected ranges for Inhibin A were calculated using 95% non-parametric estimation using Analyse-It® for Microsoft Excel.
The values represented in the graph and the table below are maternal serum inhibin A in the second trimester.

### Change in Inhibin A Concentration with Gestational Age

<table>
<thead>
<tr>
<th>Population</th>
<th>No of specimens (n)</th>
<th>Median conc. (pg/mL)</th>
<th>Inhibin A (pg/mL) 95% CI</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td>228</td>
<td>190.02</td>
<td>162.80 to 208.20</td>
</tr>
<tr>
<td>16</td>
<td>259</td>
<td>168.20</td>
<td>154.97 to 187.21</td>
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<tr>
<td>17</td>
<td>158</td>
<td>161.95</td>
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<td>18</td>
<td>108</td>
<td>169.90</td>
<td>149.76 to 194.20</td>
</tr>
<tr>
<td>19</td>
<td>48</td>
<td>172.85</td>
<td>124.60 to 213.90</td>
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<tr>
<td>20</td>
<td>26</td>
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<td>21</td>
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</tr>
<tr>
<td>22</td>
<td>9</td>
<td>153.08</td>
<td>106.00 to 357.00</td>
</tr>
</tbody>
</table>

**Note:** It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

### Intra-Cycle Variability of E2, Inhibin B and Inhibin A

The mean ± SE concentrations of inhibin A, estradiol, and Inhibin B in 9 subjects are shown, aligned relative to the day of the mid cycle LH peak. The inhibin A levels remained low (<13 pg/mL) during the early follicular phase and rose in the late follicular phase to reach a peak concentration (99.4 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 (12.5 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:

\[ \text{Method Comparison:} \]

The Inhibin A ELISA has been compared to commercially available Inhibin A kit (Dxi) using a case-control study set of 45 cases (Down Syndrome) and 221 controls. A receiver operating characteristic (ROC) curve including Down syndrome samples indicated that the screening efficiency using the Ansh Inhibin A ELISA (AL123i) is comparable.

### Method Comparison:

The Inhibin A ELISA has been compared to commercially available Inhibin A kit (Method A) using 116 pregnant female serum samples in the range of 50-795 pg/mL.

Passing Bablok analysis of the results yielded the following Regression:

\[ \text{Inhibin A ELISA (AL-123i)} = 1.03 \times \text{(Method A)} - 14.9 \ (r=0.97; P<0.0001) \]

Receiver Operating Characteristic (ROC): The Inhibin A ELISA has been compared to commercially available Inhibin A kit (Dxi) using a case-control study set of 45 cases (Down Syndrome) and 221 controls. A receiver operating characteristic (ROC) curve including Down syndrome samples indicated that the screening efficiency using the Ansh Inhibin A ELISA (AL123i) is comparable.

### REFERENCES


This assay is intended for in vitro diagnostic use.

The Ansh Labs logo is a trademark of Ansh Labs.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.
Webster, TX 77598-4217, U.S.A.

European Representative:
RD-RatioDiagnostics GmbH
Westerbachstr.47
60489 Frankfurt
Germany

Ansh Labs consumables are being shipped with English Instructions for Use (IFUs). You may contact your local Ansh Labs sales representative or technical support organization to obtain translated IFUs.

Les consommables pour Ansh Labs sont livrés avec des instructions d'utilisation en anglais. N'hésitez pas à contacter votre société d'assistance technique ou votre représentant Ansh Labs local pour obtenir des instructions traduites.

Die Verbrauchsmaterialien von Ansh Labs werden mit englischer Gebrauchsanweisung (IFU) geliefert. Wenden Sie sich gegebenenfalls an Ihren zuständigen Vertreter von Ansh Labs oder den technischen Kundendienst, um übersetzte Gebrauchsanweisungen zu erhalten.

Los consumibles para Ansh Labs se entregan con las instrucciones de uso (IFU) en inglés. También puede ponerse en contacto con el representante local de ventas de Ansh Labs o con la empresa de asistencia técnica para obtener las IFU traducidas.
## Symbols used with Ansh Labs Assays

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