INTENDED USE

The Inhibin B enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Inhibin B in human serum and other biological fluids. This assay is intended for in vitro diagnostic use.

SUMMARY AND EXPLANATION

Inhibin B is a dimeric hormone that is composed of alpha (α) and beta B (βB) subunits. The free alpha subunits usually do not have any physiological effect. Therefore, the bioactivity of the inhibins depends on the formation of a dimeric α-β structure, and only dimeric forms of inhibins are biologically active. Inhibins are 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. Inhibin B levels have been reported in sertoli cell function (potential marker for spermatogenesis and testicular function), ovarian reserve and granulosa cell tumors.

PRINCIPLE OF THE TEST

The Inhibin B ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Inhibin B antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Inhibin B 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-enzyme 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 B in the samples and calibrators.

MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>MATERIALS REQUIRED BUT NOT PROVIDED</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL-107A - CAL-107F</td>
</tr>
<tr>
<td>CTR-107-I &amp; CTR-107-II</td>
</tr>
</tbody>
</table>

For Illustrative Purposes Only: Refer to label for exact specifications.
8. Tight fitting 12 x 75 mm tube racks.

**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 immunossay materials.
c) Handle and dispose of all reagents and material in compliance with applicable regulations.

**WARNING: Potential Biohazardous Material**

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.

**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 B 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. Inhibit B Calibrators A-F and Inhibit B Controls I & II: Tap and reconstitute Inhibit B Calibrator A-F and Inhibit B Controls I & II each with 1 mL deionized water. Solubilize, 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. Inhibit B Antibody-Biotin Conjugate Solution: The Inhibit B Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Inhibit B Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220 μL of the Concentrate in to each well.

**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.

1. Reconstitute Inhibit B Calibrator A-F and Inhibit B Controls I & II each with 1 mL deionized water. Solubilize for 10 minutes, Mix well.
2. Label the microtitration strips to be used.
3. Pipette 50 μL of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add 50 μL of the Inhibit B Assay Buffer A to each well using a repeater pipette.
5. Add 50 μL of the Inhibit B Assay Buffer B to each well using a repeater pipette.

**Note:** Samples that are hemolyzed or contain catalases are susceptible to foaming. Such foaming does not impact sample results. If such samples are present, incubate for 30 minutes without shaking at room temperature (23 ± 2°C) for foaming to subside.

6. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 2 hour at room temperature (23 ± 2°C).
7. During the last 20-30 minutes of incubation, prepare the Inhibit B Antibody-Biotin Conjugate Solution by diluting the Inhibit B Biotin Conjugate Concentrate in Inhibit B Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
8. Aspirate and wash each strip 5 times with Wash Solution (350 μL/per well) using an automatic microplate washer.
9. Add 100 μL of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
10. 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).
11. Aspirate and wash each strip 5 times with the Wash Solution (350 μL/per well) using an automatic microplate washer.
12. Add 100 μL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
13. 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).
14. Aspirate and wash each strip 5 times with the Wash Solution (350 μL/per well) using an automatic microplate washer.
15. Add 100 μL of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
16. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 8-12 min at room temperature (23 ± 2°C).

**NOTE:** Visually monitor the color development to optimize the incubation time.

17. 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 B 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 B concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.

4. Determine the Inhibin B concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Inhibin B 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 B levels in human serum and lithium heparin plasma. 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.

**QUALITY CONTROL**

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Inhibin B ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Inhibin B 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>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Calibrators</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>B1, B2</td>
<td>A</td>
<td>0.085</td>
<td>12.7</td>
</tr>
<tr>
<td>C1, C2</td>
<td>B</td>
<td>0.176</td>
<td>34</td>
</tr>
<tr>
<td>D1, D2</td>
<td>C</td>
<td>0.527</td>
<td>129</td>
</tr>
<tr>
<td>E1, E2</td>
<td>D</td>
<td>1.595</td>
<td>446</td>
</tr>
<tr>
<td>F1, F2</td>
<td>Blank</td>
<td>3.432</td>
<td>1390</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**

**Limit of Detection (LoD):**

The limit of detection in the assay as calculated by the interpolation of mean plus two standard deviation of 24 replicates of calibrator A (0 pg/mL) and calibrator B (12.7 pg/mL) is 1.6 pg/mL.

**Limit of Quantitation (LoQ):**

The estimated minimum Inhibin B dose achieved at 20% total imprecision is 4.6 pg/mL. The value was determined by processing seven samples in the range of 2.95-364.12 pg/mL with seven runs in quadruplets (n=28).

**Imprecision:**

Reproducibility of the Inhibin B assay was determined in a study using two serum pools and two kit controls. The study included a total of 20 assays, four replicates of each per assay (n=78-80). Representative data were calculated based on NCCLS EP5-A guidelines and are presented in the following table.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Conc.</th>
<th>Within Run</th>
<th>Between Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(pg/mL)</td>
<td>SD</td>
<td>%CV</td>
<td>SD</td>
</tr>
<tr>
<td>Pool</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control I</td>
<td>12.998</td>
<td>2.690</td>
<td>14.0%</td>
<td>1.316</td>
</tr>
<tr>
<td>Pool 2</td>
<td>13.456</td>
<td>3.285</td>
<td>18.0%</td>
<td>1.342</td>
</tr>
<tr>
<td>Pool</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control I</td>
<td>308.103</td>
<td>11.222</td>
<td>3.7%</td>
<td>9.394</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogenous Conc. (pg/mL)</th>
<th>Expected Conc. (pg/mL)</th>
<th>Observed Conc. (pg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.86</td>
<td>112.44</td>
<td>106.53</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>66.97</td>
<td>127.78</td>
<td>127.75</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>144.88</td>
<td>203.19</td>
<td>209.59</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>159.89</td>
<td>216.28</td>
<td>215.28</td>
<td>99</td>
</tr>
</tbody>
</table>

**Linearity:**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>Observed Conc. (pg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:2</td>
<td>659.2</td>
<td>674.6</td>
</tr>
<tr>
<td>1</td>
<td>1:4</td>
<td>329.6</td>
<td>310.8</td>
</tr>
<tr>
<td>1</td>
<td>1:8</td>
<td>164.8</td>
<td>173.3</td>
</tr>
<tr>
<td>1</td>
<td>1:16</td>
<td>82.4</td>
<td>84.5</td>
</tr>
<tr>
<td>1</td>
<td>1:32</td>
<td>41.2</td>
<td>47.1</td>
</tr>
</tbody>
</table>

**CAUTION:** Refer to package insert included with the product for exact specifications.
Analytical Specificity:
This monoclonal antibody pair used in the assay detects Inhibin B. 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 and triglycerides were added at a greater than two folds of their physiological concentration to control sample, Inhibin B concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP7-P.

Expected Value:
The expected ranges for Inhibin B were calculated using 95% non-parametric estimation for Male Samples and 97.5% non-parametric estimation for Female Samples using Analyse-It® for Microsoft Excel.

Method Comparison:
The Inhibin B ELISA has been compared to commercially available Inhibin B kit (Method A) using 97 random male and female serum samples in the range of 10-174 pg/mL.

Passing Bablok analysis of the results yielded the following Regression:

Inhibin B ELISA (AL-107) = 0.93 (Method A) + 1.08
(r=0.97; P<0.0001)
REFERENCES


This assay is intended for in vitro diagnostic use. Not for sale in the U.S.A.

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

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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.

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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.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
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<td>Consult instructions for use</td>
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<td>Consulter les instructions d'utilisation</td>
<td>Consulte las instrucciones de uso</td>
<td>Consultare le istruzioni per l'uso</td>
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<td>☭</td>
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<td>CE-Konfirmitäts-kennzeichnung</td>
<td>Conformité aux normes européennes</td>
<td>Conformidad europea</td>
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<td>In-vitro-Diagnostikum</td>
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<td>Numero di lotto</td>
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<td>Contenuto</td>
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<td></td>
<td>Volume/No.</td>
<td>Volume / No.</td>
<td>Volumen/Anzahl</td>
<td>Volumen/Número</td>
<td>Volume/Quantità</td>
</tr>
</tbody>
</table>

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