# Total Inhibin ELISA

**RUO**

## AL-134

### INTENDED USE

The Total Inhibin enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Total inhibin (Inhibin A, Inhibin B and Inhibin alpha subunit) in human serum, plasma and other biological fluids. This kit is intended for laboratory **Research Use Only** and is not for use in diagnostic or therapeutic procedures.

### 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 the two distinct chains ($\alpha$ and $\beta$), linked by disulfide bridges. Higher molecular weight forms, with precursor forms of the $\alpha$-subunit, also occur in follicular fluid and serum. In addition, free $\alpha$-subunit forms, unassociated with a $\beta$-subunit, and lacking inhibin bioactivity, are also present. Measurements of Inhibins are shown to be useful in studying its role in human reproductive physiology, spermatogenesis, testicular function, ovarian reserve, granulosa cell tumors and mucinous tumors.

### PRINCIPLE OF THE TEST

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

### MATERIALS SUPPLIED

**CAL-134A Total Inhibin Calibrator A (Lyophilized)**

One vial, labeled A, containing 0 pg/mL Inhibin in animal sera and a non-mercury preservative. Store unopened at 2 to 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 to 8°C. For longer storage after reconstitution, aliquot, and freeze at -20°C or colder for up to one year.

**CAL-134B - CAL-134F Total Inhibin Calibrators B thru F (Lyophilized)**

Five vials, labeled B-F, containing concentrations of approximately 10-500 pg/mL Inhibin A in animal sera and a non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 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 to 8°C. For longer storage after reconstitution, aliquot, and freeze at -20°C or colder for up to one year.

***Standardization Note:***

1. The Total Inhibin calibrators are traceable to the WHO International Standard, Inhibin A, Human, Recombinant, NIBSC code: 91/624 (Version 3.0, Dated 25/01/2008)
   Total Inhibin Calibrators = 2.0 (Recombinant Human Inhibin A WHO 91/624, Version 3.0, Dated 25/01/2008)
2. The Total Inhibin calibrators are traceable to the WHO Reference Reagent, Inhibin B, Human, NIBSC code: 96/784 (Version 2.0, Dated 17/01/2008)
   Total Inhibin Calibrators = 0.29 (Human Inhibin B WHO 96/784, Version 2.0, Dated 17/01/2008)

**CTR-134-I & CTR-134-II Total Inhibin 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 to 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 to 8°C. For longer storage after reconstitution, aliquot, and freeze at -20°C or colder for up to one year.

**PLT-134 Total Inhibin antibody Coated Microtitration Strips**

One stripholder, containing 12 strips and 96 microtitration wells with anti-Inhibin (alpha) 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-207A Inhibin B Assay Buffer A**

One bottle, 8 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

**ASB-207B Inhibin B Assay Buffer B**

One bottle, 8 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

**BCC-134 Total Inhibin Biotin Conjugate Concentrate**

One vial, 0.4 mL containing detection antibody biotin conjugate in a protein-based buffer with a non-mercury preservative. Dilute prior to use in Inhibin B Conjugate diluent. Store at 2-8°C until expiration date.

**CND-207 Inhibin B 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-134 Total Inhibin Streptavidin-Enzyme Conjugate—Ready-to-Use (RTU)**

One amber 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 to 8°C until expiration date.

**STP-100 Stopping Solution**

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 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 to 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 research use only.**

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 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[1].

**WARNING: Potential Chemical Hazard**

Some reagents in this kit contain Pro-Clean 400 and Sodium Azide[2] 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 Total Inhibin 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 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. **Total Inhibin Calibrators A-F**: Tap and reconstitute Total Inhibin Calibrators A-F 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 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. **Total Inhibin Antibody-Biotin Conjugate Solution:** The Total Inhibin Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Inhibin 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 11 mL of the diluent.

## ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature 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 Total Inhibin Calibrators A-F and Total Inhibin Controls I & II each with **1.0 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 Inhibin B Assay Buffer A to each well using a repeater pipette.
5. Add 50 **µL** of the Inhibin B Assay Buffer B to each well using a repeater pipette.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **2 hours** at room temperature.
7. During the last **20-30 minutes** of incubation, prepare the Total Inhibin Antibody-Biotin Conjugate Solution by diluting the Total Inhibin Biotin Conjugate Concentrate in Inhibin 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 Washing 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.
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.
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 precision pipette. Avoid exposure to direct sunlight.
16. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **10-12 min** at room temperature.
    ***NOTE**: Visually monitor the color development to optimize the incubation time.*
17. Add **100 µL** of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm.**
    ***NOTE**: While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "**Blank**".*

## RESULTS

***NOTE:** The results in this package insert were calculated by plotting the data on a log vs. log scale using a cubic regression curve-fit. Other data reduction methods may give slightly different results.*

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

## LIMITATIONS

The reagents supplied in this kit are optimized to measure Total Inhibin 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[4].

The Total Inhibin 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.
- Total Inhibin ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Total Inhibin 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

| Well Number | Well Contents Calibrators | Mean OD | Conc (pg/mL) |
|---|---|---|---|
| **A1, A2** | A | 0.06 (**Blank**) | 0 |
| **B1, B2** | B | 0.084 | 8.3 |
| **C1, C2** | C | 0.22 | 24.8 |
| **D1, D2** | D | 0.66 | 82.5 |
| **E1, E2** | E | 1.53 | 220 |
| **F1, F2** | F | 3.26 | 525 |

***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 16 replicates of calibrator A (0 pg/mL) and calibrator B 8.3 pg/mL) is 0.94 pg/mL.

### Imprecision:

Reproducibility of the Total Inhibin ELISA assay was determined in a study using two controls and three serum samples. The study included a total of 9 assays, two replicates of the controls and samples per assay (n=18). Representative data were calculated based on CLSI EP5-A2 guidelines and are presented in the following table.

| Sample | Mean (pg/mL) | Within run | | Between run | | Total | |
|---|---|---|---|---|---|---|---|
| | | SD | CV | SD | CV | SD | CV |
| Control I | 20.6 | 0.8 | 4.1% | 0.4 | 2.0% | 0.9 | 4.5% |
| Control II | 69.8 | 2.1 | 3.0% | 1.8 | 2.6% | 2.7 | 3.9% |
| Sample 1 | 47.7 | 2.5 | 5.3% | 4.1 | 8.5% | 4.8 | 10.0% |
| Sample 2 | 98.4 | 1.0 | 1.0% | 2.4 | 2.5% | 2.7 | 2.7% |
| Sample 3 | 174.5 | 5.3 | 3.0% | 8.8 | 5.0% | 10.2 | 5.9% |

### Linearity:

Multiple dilutions of the two serum samples and Calibrator F containing various Inhibin levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

| Sample ID | Dilution factor (1 in X) | Expected Value in pg/mL | Observed Value in pg/mL | %Recovery | Average %Recovery |
|---|---|---|---|---|---|
| Calibrator F | Neat | 540.0 | | | 92% |
| | 2 | 270.0 | 234.3 | 87% | |

| | | | | | |
|---|---|---|---|---|---|
| | 4 | 135.0 | 120.9 | 90% | |
| | 8 | 67.5 | 60.2 | 89% | |
| | 16 | 33.8 | 31.7 | 94% | |
| | 32 | 16.9 | 16.8 | 100% | |
| Sample-1 | Neat | 96.7 | | | 106% |
| | 2 | 48.3 | 47.4 | 98% | |
| | 4 | 24.2 | 25.4 | 105% | |
| | 8 | 12.1 | 13.2 | 110% | |
| | 16 | 6.0 | 6.8 | 113% | |
| Sample-2 | Neat | 120.9 | | | 103% |
| | 2 | 60.5 | 58.6 | 97% | |
| | 4 | 30.2 | 30.5 | 101% | |
| | 8 | 15.1 | 16.5 | 109% | |
| | 16 | 7.6 | 7.9 | 105% | |

**Recovery:**

Known amounts of Recombinant Inhibin A (Ansh Labs Part No. AI035) were added to three serum samples containing different levels of endogenous Inhibin. The concentration of Inhibin was determined before and after the addition of exogenous Inhibin A and the percent recovery was calculated.

| Sample ID | Endogenous Value in pg/mL | Expected in pg/mL | Observed in pg/mL | %Recovery |
|---|---|---|---|---|
| 1 | 127.7 | 133.4 | 134.1 | 101% |
| | | 139.0 | 140.1 | 101% |
| | | 144.7 | 142.4 | 98% |
| 2 | 44.6 | 54.4 | 55.8 | 103% |
| | | 64.2 | 65.8 | 102% |
| | | 74.0 | 74.7 | 101% |
| 3 | 50.9 | 60.4 | 64.0 | 106% |
| | | 69.9 | 72.8 | 104% |

Known amounts of Recombinant Inhibin B (Ansh Labs Part No. BI043) were added to five serum samples containing different levels of endogenous Inhibin. The concentration of Inhibin was determined before and after the addition of exogenous Inhibin B and the percent recovery was calculated.

| Sample ID | Endogenous Value in pg/mL | Expected in pg/mL | Observed in pg/mL | %Recovery |
|---|---|---|---|---|
| 1 | 127.2 | 138.2 | 127.2 | 92% |
| | | 149.1 | 139.2 | 93% |
| | | 160.1 | 151.4 | 95% |
| 2 | 12.7 | 29.4 | 33.4 | 114% |
| | | 46.1 | 52.5 | 114% |
| | | 62.7 | 71.1 | 113% |
| 3 | 96.3 | 108.8 | 109.2 | 100% |
| | | 121.3 | 125.0 | 103% |
| | | 133.8 | 145.3 | 109% |
| 4 | 43.3 | 58.5 | 62.1 | 106% |
| | | 73.6 | 81.0 | 110% |
| | | 88.8 | 100.6 | 113% |
| 5 | 54.5 | 69.1 | 71.4 | 103% |
| | | 83.7 | 88.3 | 105% |
| | | 98.3 | 109.2 | 111% |

**Analytical Specificity:** The antibody pair used in the Total Inhibin assay detects Inhibin A and Inhibin B and is targeted to detect Inhibin alpha subunit. There is no cross-reactivity detected with Activin A, Activin B, Activin AB, Myostatin, Follistatin 288, Follistatin 315, alpha-2-Macroglobulin, LH, FSH and hAMH (Pro+Mature). The antibody pair used in Total Inhibin assay detects Bovine, Equine, Ovine, Porcine, Canine, and Mouse samples and does not detect Goat and Rabbit samples.

**Interference:**

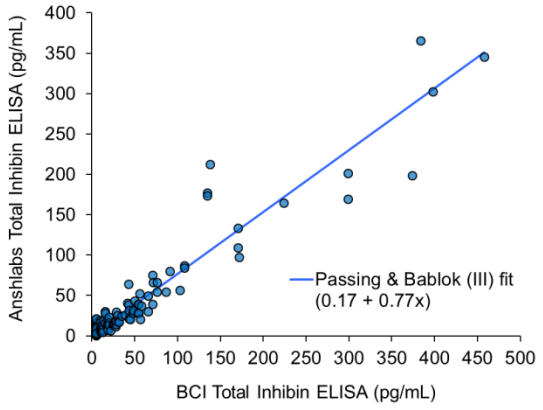
When hemoglobin, biotin, intralipids and bilirubin were added at a concentration greater than two folds of their physiological concentration to control sample, average Total Inhibin concentration were within ± 15% of the control as represented in the following table.

| Interferent | Interferent Dose | Analyte Conc. (pg/mL) | Spiked Sample Value (pg/mL) | % Difference |
|---|---|---|---|---|
| Hemoglobin | 1 mg/mL | 105.7 | 97.7 | -7.6 |
| | 0.5 mg/mL | 114.5 | 108.8 | -5.0 |
| | 0.1 mg/mL | 124.0 | 122.9 | -0.9 |
| Hemoglobin | 1 mg/mL | 44.9 | 39.4 | -12.3 |
| | 0.5 mg/mL | 47.0 | 43.9 | -6.6 |
| | 0.1 mg/mL | 49.7 | 48.4 | -2.8 |
| Biotin | 1200 ng/mL | 102.4 | 104.5 | 2.1 |
| | 600 ng/mL | 113.8 | 114.2 | 0.4 |
| | 200 ng/mL | 119.0 | 117.5 | -1.3 |
| Biotin | 1200 ng/mL | 43.5 | 42.7 | -1.9 |
| | 600 ng/mL | 46.7 | 46.6 | -0.3 |
| | 200 ng/mL | 49.9 | 49.9 | 0.1 |
| Intralipids | 20 mg/mL | 106.5 | 107.1 | 0.5 |
| | 10 mg/mL | 116.3 | 116.8 | 0.4 |
| | 5 mg/mL | 121.4 | 121.0 | -0.3 |
| Intralipids | 20 mg/mL | 44.9 | 44.7 | -0.5 |
| | 10 mg/mL | 45.5 | 45.7 | 0.3 |
| | 5 mg/mL | 47.9 | 47.9 | 0.1 |
| Bilirubin | 0.66 mg/mL | 78.2 | 81.2 | 3.8 |
| | 0.2 mg/mL | 112.4 | 112.1 | -0.3 |
| Bilirubin | 0.66 mg/mL | 31.3 | 32.1 | 2.5 |
| | 0.2 mg/mL | 44.9 | 44.4 | -1.0 |

**Method Comparison:**

The Total Inhibin ELISA has been compared to known values of a commercial Total Inhibin kit using 153 female serum samples in the range of 5-458 pg/mL. Passing Bablok analysis of the results yielded the following Regression:

Ansh Labs Total Inhibin ELISA (AL-134) = 0.77 (BCI Total Inhibin ELISA) +0.17 (rs=0.88; P<0.0001)



## REFERENCES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This assay is intended for research use only.



Manufactured by:
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