

Mouse Inhibin B (INH-B) ELISA Kit

Catalog No. CSB-E08151m

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **mouse INH-B** concentrations in **serum, plasma and other biological fluids**.
- **Expiration date** six months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

Inhibin is a peptide that is an inhibitor of FSH synthesis and secretion, and participates in the regulation of the menstrual cycle. Inhibin contains an alpha and beta subunit linked by disulfide bonds. Two forms of inhibin differ in their beta subunits (A or B), while their alpha subunits are identical. Inhibin belongs to the transforming growth factor- β (TGF- β) superfamily. In women, FSH stimulates the secretion of inhibin from the granulosa cells of the ovarian follicles in ovary. In turn, inhibin suppresses FSH. Inhibin secretion is diminished by GnRH, and enhanced by insulin-like growth factor-1 (IGF-1). Inhibins have been defined based on their activity of suppressing pituitary gonadotropin secretion. Thus, the serum concentrations of Inhibin B and FSH are inversely correlated, and at low serum levels of Inhibin B, FSH concentration goes up. Inhibin B serum concentration increases gradually in the follicular phase to a broad peak at 7 days prior to the LH surge, and may constitute the limiting factor for the duration of the inter-cycle FSH rise. A decrease and a second peak follow this peak one-day past the LH surge. Most of the serum Inhibin B concentration originates from large follicles, since such follicles secrete 10 fold higher

concentrations than do the small follicles. Inhibin B concentrations drop to a very low concentration at the luteal phase, and is undetectable during pregnancy.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are then added to the appropriate microtiter plate wells with a Horseradish Peroxidase (HRP)-conjugated INH-B and antibody preparation specific for INH-B, and incubated. Then substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of INH-B in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

0.8 pg/ml-200 pg/ml. The standard curve concentrations used for the ELISA's were 200 pg/ml, 50 pg/ml, 12.5 pg/ml, 3.1 pg/ml, 0.8 pg/ml.

SPECIFICITY

This assay recognizes mouse INH-B. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of mouse INH-B is typically less than 0.2 pg/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard	5 x 0.5 ml
HRP-Conjugate	1 x 6ml
Antibody	1 x 6 ml
Wash Buffer	1 x 15 ml (20xconcentrate)
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml

Standard	S1	S2	S3	S4	S5
Concentration(pg/ml)	0.8	3.1	12.5	50	200

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Set a Blank without any solution. Add 50µl of Standard or Sample per well.

2. Add 50µl of **HRP-Conjugate** and 50µl of **Antibody** to each well. Not to Blank well!
3. Cover with the adhesive strip. Incubate for 1 hour at 37°C.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the INH-B concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.

- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.