7. During the last **20-30 minutes** of incubation, prepare the Inhibin B Antibody-Biotin Conjugate Solution by diluting the Inhibin B 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 Substrate Solution to each well using a repeater pipette.
16. Incubate the wells, shaking at **600-800 rpm** on an orbital microplate shaker for 4±1 minute at room temperature. Avoid exposure to direct sunlight.
17. Read the light output of the solution in the wells within 10 minutes, using a microplate luminometer.
    NOTE: *While reading the RLU'S of the well, it is necessary to program the 0 calibrator as "BLANK."*

ALTERNATIVE ASSAY PROCEDURE

1. Reconstitute Inhibin B Calibrator A-F and Inhibin B Controls I & II each with **1 mL** deionized water. Solubilize for **10 minutes**, Mix well.
2. For each unknown serum sample, calibrators and controls label one 12 X 75 culture tubes.
3. Pipette **75 µL** of the Calibrator, Controls and samples to the pre-labeled tube.
4. Add **75 µL** of the Inhibin B Assay Buffer A to each the pre-labeled tube using a repeater pipette.
5. Add **75 µL** of the Inhibin B Assay Buffer B to each pre-labeled tube using a repeater pipette and vortex well.
6. Place the tubes in a tight fitting tube rack and incubate the tubes, shaking at a slow speed **(100-200 rpm)** at room temperature for **30 minutes**.
7. The pre-mixed samples are now ready for analysis.
8. Label the microtitration strips to be used.
9. Pipette **150 µL** of the pre-mixed samples from **step 7** to the appropriate wells.
10. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **2 hour** at room temperature.
11. During the last **20-30 minutes** of incubation, prepare the Inhibin B Antibody-Biotin Conjugate Solution by diluting the Inhibin B Biotin Conjugate Concentrate in Inhibin B Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.
12. Aspirate and wash each strip **5 times** with Washing Solution **(350 µL/per well)** using an automatic microplate washer.
13. Add **100 µL** of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
14. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **1 hour** at room temperature.
15. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
16. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
17. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature.
18. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
19. Add **100 µL** of the Substrate Solution to each well using a repeater pipette.
20. Incubate the wells, shaking at **600-800 rpm** on an orbital microplate shaker for 4±1 minute at room temperature. Avoid exposure to direct sunlight.
21. Read the light output of the solution in the wells within 10 minutes, using a microplate luminometer.
    NOTE: *While reading the RLU'S of the well, it is necessary to program the 0 calibrator as "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 RLU for each calibrator, Control, or Unknown.
2. Plot the log of the mean RLU 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.
3. Determine the Inhibin B concentrations of the Controls and unknowns from the calibration curve by matching their mean RLU readings with the corresponding Inhibin B 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 Inhibin B 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].

QUALITY CONTROL

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

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean RLU	Conc (pg/mL)
	Calibrators		
A1, A2	A	0.318 (**Blank**)	0
B1, B2	B	0.437	14
C1, C2	C	1.993	42
D1, D2	D	9.638	125
E1, E2	E	45.841	415
F1, F2	F	158.287	1344

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