DATA SHEET

Fish Insulin (INS)ELISA Kit

Catalog No. CSB-E12123Fh
(96 tests)

This immunoassay kit allows for the in vitro rapid detection of fish INS 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

Insulin is a peptide hormone composed of 51 amino acid residues and has a molecular weight of 5808 Da. It is produced in the islets of Langerhans in the pancreas. Insulin's structure varies slightly between species of animal. Insulin has extensive effects on both metabolism and several other body systems (eg, vascular compliance). Insulin causes most of the body's cells to take up glucose from the blood (including liver, muscle, and fat tissue cells), storing it as glycogen in the liver and muscle, and stops use of fat as an energy source. When insulin is absent (or low), glucose is not taken up by most body cells and the body begins to use fat as an energy source (ie, transfer of lipids from adipose tissue to the liver for mobilization as an energy source).
As its level is a central metabolic control mechanism, its status is also used as a control signal to other body systems (such as amino acid uptake by body cells). It has several other anabolic effects throughout the body.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. A antibody specific to INS has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with biotin conjugated INS and incubated. A competitive inhibition reaction is launched between INS (Standards or samples) and Biotin-conjugated INS with the pre-coated antibody specific for INS. The more amount of INS in samples, the less antibody bound by Biotin-conjugated INS. Then Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of INS in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

The standard curve concentrations used for the ELISA's were 1600 pg/ml, 800 pg/ml, 400pg/ml, 200pg/ml, 100pg/ml.

SPECIFICITY

This assay recognizes INS. No significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standards (S1-S5)	5
HRP-avidin	1 x 6 ml
Conjugate	1 x 6 ml
Wash Buffer (20×concentrate)	1 x 15 ml
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml

Standard	S1	S2	S3	S4	S5
Concentration(pg/ml)	100	200	400	800	1600

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 with desiccants to minimize exposure to damp air. 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.

TECHNICAL HINTS

1. Bring all reagents and plate to room temperature for at least 30 minutes before use. Unused wells need store at 2-8°C and avoid sunlight.
2. Wash Buffer If crystals have formed in the concentrate, warm 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.
3. 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.
4. 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.
5. 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.
6. 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.
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.
- Plasma Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. 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 well without any solution. Add 50µl of Standard or Sample per well. Standard need test in duplicate.

2. Add 50µl of Biotin-conjugate INS to each well (not to Blank well), Mix well and then incubate for 1 hour at 37°C.
3. Fill each well with Wash Buffer (about 200µl), stay for 10 seconds and Spinning. Repeat the process for a total of three washes. 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.
4. Add 50µl of HRP-avidin to each well. Incubate for 30mins at 37°C.
5. Repeat the aspiration and wash five times as step 4.
6. Add 50µl of Substrate A and Substrate B to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
7. Add 50µl of Stop Solution to each well.
8. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, Blank, and sample and subtract the optical density of Blank. 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 INS 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 Standard Diluent, 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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium