Insulin （INS）
ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for Insulin（INS） (Enzyme-Linked ImmunoSorbent Assay , ELISA).It is used in quantitative tests for Insulin in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Insulin is a kind of protein hormone which is produced and reprocessed by the pancreas islets beta-cell. It is composed of 51 amino acids α, β, Molecular weight of 5734D [1]. Insulin and animal insulin in different species are slightly different, and the structure of porcine insulin is most similar to that of human, except that the last amino acid on the β chain is different, so there is a clear cross reaction in both immunoassays.

Insulin is to promote the synthesis of hormones, the main role is to promote the oxidation of glucose and glycogen production, inhibition of glycogen mutation, so as to maintain blood sugar constant. Insulin deficiency, the blood glucose concentration increased, can exceed the renal sugar threshold, the occurrence of dependence on insulin diabetes [2].

PRINCIPLE

INS kit uses a “sandwich principle”, Enzyme-linked immunological sorbent assay. To measure INS levels in serum, plastic wells coated with a monoclonal antibody of INS are supplied in the kit. After the patient’s specimen and another mono-antibody labelled with HRP are added, INS, is fixed to the solid phase antibody and creating a HRP-antibody— INS—antibody “sandwich”. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is proportional the concentration of the patient sample.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the INS procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2~8℃. Specimens held for a longer time can be frozen at –20℃ and avoid repeated freeze melting.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with a anti-INS antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2-8℃ until expiration date.
2. HRP Conjugate: 1 vial of 6ml, Ready to use. Store at 2~8℃ until expiration date.
3. Calibrator: 6 vials of 1ml, Ready to use. Labeled with S0 to S5 and the concentration of INS is 0, 10, 20, 40, 80, 160 mIU/L. Store at 2~8℃ until expiration date.
4. Control: 2 vials of 1ml, Ready to use. The concentration of low value is 11.6~19.2mIU/L. The concentration of high value is 54.4~90.6mIU/L. Store at 2~8℃ until expiration date.
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
8. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2~8℃ until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml wash buffer. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with water and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
4. Storage. The whole kit should be stored at 2~8℃ for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash buffer by diluting Wash Concentrate 20-fold with deionized water. The
diluted wash buffer is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the calibrators and controls should set duplicate.
2. Dispense 50 µl of calibratorsas/controls/samples into wells.
3. Dispense 50 µl of HRP Conjugate to each well.
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 60 minutes.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 50 µl of chromogen A to each well.
7. Dispense 50 µl of chromogen B to each well.
8. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
9. Dispense 50 µl of stopping solution to each well and mix completely.
10. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: log OD=B*log [concentration]+A

PERFORMANCE CHARACTERISTICS

1. Expected value

Each laboratory should establish its own range of normal values. The value give below are only indicative. For information, the range of basal insulin levels in normal subjects was 3.1~25.3 mIU/L. One hour after meal: 35~210 mIU/L.

2. Sensitivity

The detection limit of the assay is approximately 2 mIU/L.

3. Precision

Interassay≤15%

Intraassay≤15%

4. Specificity: No cross reaction with C-P, Pro-INS.

CODIGO : RSET104-3