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U Mass - Insulin

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We use this protocol and it's working

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Abstract

Summary:

The insulin enzyme-linked immunosorbent assay (ELISA) is a standard immunoassay for the detection of insulin levels in serum samples. This experiment uses a 96-well plate coated with anti-insulin antibodies and developed with a secondary anti-insulin antibody linked to horseradish peroxidase. The ELISA is developed with 3,3',5,5'-Tetramethylbenzidine (TMB), a chromogenic substrate and read on an automated plate spectrophotometer at 450 nm. Insulin ELISA may be processed with normal or ultra-sensitive assay kit depending on the expected range of sample concentrations. Serum insulin levels are affected by pancreatic β-cell function and insulin sensitivity. Serum insulin levels are elevated in obesity and insulin resistance.

Materials

MATERIALS

- Insulin Microplate (96 wells) **Alpco Catalog #**80-INSMSU-E01(Kit)
- Zero Standard **Alpco**
- Standards (A-G)* (0.025 0.09 0.188 0.5 1.25 3.75 6.9 ng/ml) **Alpco**
- Control Levels 1 2 and 3 **Alpco**
- Conjugate Stock **Alpco**
- Conjugate Buffer **Alpco**
- Wash Buffer Concentrate **Alpco**
- TMB Substrate **Alpco**
- Stop Solution **Alpco**
- Plate Sealers **Alpco**

Note:
Alpco Diagnostics RRID:SCR_013569
80-INSMSU-E01, Cite this**(Alpco Diagnostics Cat# 80-INSMSU-E01, RRID:AB_2792981)**

Additional Items

1. Precision pipettes for dispensing 5, 25, 75, and 100 µL (with disposable tips)
2. Repeating or multi-channel pipette for dispensing 75 and 100 µL
3. Volumetric containers and pipettes for reagent preparation
4. Distilled or deionized water for reagent preparation
5. Microplate washer or wash bottle
6. Microplate shaker capable of 700-900 rpm
7. Microplate reader with 450 nm filter
8. Vortex for sample preparation
9. Precision pipettes for dispensing 5, 25, 75, and 100 µL (with disposable tips)
10. Repeating or multi-channel pipette for dispensing 75 and 100 µL
11. Volumetric containers and pipettes for reagent preparation
12. Distilled or deionized water for reagent preparation

Reagent Preparation:

Reagent 1:
Preparation of Conjugate Buffer:
1. Conjugate Stock may be diluted with 10 parts Conjugate Buffer.

2. To prepare enough Working Strength Conjugate for one complete microplate, dilute 0.9 mL of Conjugate Stock (11x) with 9 mL of Conjugate Buffer.

Reagent 2:
Preparation of Wash Buffer Concentrate:
1. Wash Buffer Concentrate may be diluted with 20 parts distilled water.

2. To prepare Working Strength Wash Buffer, dilute 20 mL of Wash Buffer Concentrate (21x) with 400 mL of deionized water.

3. Working Strength Wash Buffer is stable for 4 weeks at room temperature (18~25°C).

Reagent 3:
Preparation of Controls:
1. Controls (Levels 1, 2, and 3) are provided in a lyophilized form. Refer to the Certificate of Analysis provided with each kit for the appropriate volume of deionized water for reconstitution.

2. Close the vial with the rubber stopper and cap.

3. Gently swirl the vial, and wait 30 minutes prior to use.

4. The contents of the vial should be in solution with no visible particulates.

5. The reconstituted controls are stable for 7 days stored at 2~8°C.

6. If desired, the controls can be stored at ≤ -20°C in aliquots for up to 6 months. The controls should not be repeatedly frozen and thawed.

Before start

Notes:

√ Serum and plasma samples may be used for this measurement with no requirement for dilution or sample treatment. Sample dilution may be required based on expected and actual concentrations.

√ In order to minimize potential time-associated sample drift, it is recommended to thoroughly vortex each sample before use and to perform each pipetting action without pause.

√ Samples can be stored at 2~8°C for 24 hours prior to measurement. For longer storage, a temperature of less than -20°C is recommended. Avoid repeated freeze/thaw cycles.

1 The microplate should be equilibrated to room temperature prior to opening the foil pouch.

2 Assign microplate strips for a duplicate measurement for the standards, controls, and samples. The remaining microplate strips can be stored at 2~8°C in the tightly sealed foil pouch containing the desiccant.

3 Pipette 5 or 25 µL of each standard, control, and sample into respective wells.

4 See Reagent Preparation and Certificate of Analysis for control reconstitution instructions.

5 For a 5 µL sample volume, use standard concentrations of 0.188, 0.5, 1.25, 3.75, and 6.9 ng/mL (C-G) and Control Levels 2 and 3.

6 For a 25 µL sample size, use standard concentrations of 0.025, 0.9, 0.188, 0.5, and 1.25 ng/mL (A-E) and Control Levels 1 and 2.

7 Pipette 75 µL of Working Strength Conjugate (see Reagent Preparation) into each well.

8 Cover microplate with a plate sealer, and incubate for 2 hours at room temperature while shaking at 700~900 rpm using a microplate shaker.

9 Decant the contents of the wells, and wash the microplate 6x with 350 µL of Working Strength Wash Buffer per well (see Reagent Preparation) using a microplate washer.

10 Alternatively, fill the wells with Working Strength Wash Buffer using a wash bottle equipped with a wash nozzle. It is not recommended to use a multichannel pipette.

11 Wash buffer must be dispensed with adequate and equal force in order to properly wash the wells.

12 Between washes, invert the microplate to discard the liquid and firmly tap the inverted microplate on absorbent paper towels.

13 After the final wash, remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels.

14 Pipette 100 µL of TMB Substrate into each well.

15 Cover microplate with a plate sealer and incubate for 30 minutes at room temperature while shaking at 700~900 rpm on a microplate shaker.

16 Pipette 100 µL of Stop Solution into each well, and gently shake the microplate to mix the contents.

17 Remove any bubbles before proceeding with the next step.

18 Place the microplate in a microplate reader capable of reading the absorbance at 450 nm.

19 The microplate should be analyzed immediately after the addition of the Stop Solution and within 30 minutes.