Human Proinsulin ELISA Kit

Catalog number: EK7001

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Catalog Number: EK7001

Size: 96wells/kit

Sample Type: Serum

Sensitivity: 2.6 pmol/L

Assay Range: 2.6-66 pmol/L

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

Proinsulin EIA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Proinsulin molecule. An aliquot of patient sample containing endogenous Proinsulin is incubated in the coated wells. After washing off the samples in a second step an enzyme conjugate, which is an anti-Proinsulin antibody conjugated with horseradish peroxidase is incubated in the wells. After incubation the unbound conjugate is washed off with wash solution. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Proinsulin in the patient sample.

Kit Components

Description	Quantity
Microwell coated with anti Pro-insulin Antibody	12x8x1 Microwells
Proinsulin Standards	6 vials (ready to use) 1ml
Pro-Insulin Enzyme Conjugate 11X	1 vial 1.2 ml
TMB Substrate	1 bottle (ready to use) 14ml
Stop Solution	1 bottle (ready to use) 14ml
Wash concentrate 40X	1 bottle (ready to use) 30ml
Sample Diluent	1 vial (ready to use) 2 mL
Conjugate Diluent	1 bottle (ready to use) 12 mL
control (low & high)	2 ml
Assay Buffer	1 bottle (ready to use) 12 mL

Materials Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

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WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.

2. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that standards, control and serum samples be run in duplicate.

6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.

2. Typically, specimens may be stored refrigerated at (2°C to 8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.

3. Avoid multiple freeze-thaw cycles.

4. Prior to assay, frozen sera should be completely thawed and mixed well.

5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (30 mL, 40X) to 475 mL of distilled or deionized water. Store at RT.

Dilute the concentrated Enzyme Conjugate in the Conjugate Diluent (100 µl Enzyme Conjugate + 1000 µl Conjugate Diluent). For every well you need 100 µl diluted Enzyme Conjugate. The diluted Enzyme Conjugate is stable for 24 h at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Secure the desired number of coated Microtiter wells in the holder.

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2. Dispense 100 µl of Proinsulin Standards control and samples into appropriate wells.

3. Dispense 100 µl of Assay buffer into each well.

4. Mix thoroughly for 10 seconds. It is important to achieve a complete mixing in this step.

5. Cover the plate with a plate sealer and incubate overnight (16-24 hours) at 4° C in a humidity chamber.

6. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (350 µl per well). Strike the Wells sharply on absorbance paper to remove residual droplets.

7. Dispense 100 µl of diluted Enzyme-Conjugate into each well.

8. Mix thoroughly for 10 seconds. It is important to achieve a complete mixing in this step.

9. Incubate for 60 minutes at room temperature without agitation.

10. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (350 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.

11. Add 100 µl of Substrate Solution to each well at timed intervals.

12. Incubate for 30 minutes at room temperature.

13. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well.

14. Read the OD at 450±10 nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in pmol/l with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

2. Calculate the average absorbance values for each set of reference standards, controls and patient samples.

3. Using the mean absorbance value for each sample determine the corresponding concentration of Proinsulin in pmol/l from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Any diluted samples must be further converted by the appropriate dilution factor. If in an initial assay, a specimen is found to contain more proinsulin than the upper limit of the standard curve, the specimens must be diluted with Sample diluent.

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