Human Insulin antibody ELISA Kit

Enzyme Immunoassay for the quantification of human Insulin antibody in serum or plasma.

Catalog number: ARG80395

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INTRODUCTION

Type I Diabetes is mainly characterized by limited or fully missing secretion of the hormone insulin. Morphological studies demonstrated a destruction of the beta cells of the so-called Langerhans cells (islet cells) in type I diabetics. Numerous researchers described the appearance of antibodies directed against the islet cells and insulin as the causal reason for the onset of the disease.

Anti-Insulin antibodies are found in 37 percent of patients with newly detected Type I Diabetes, in 4 percent of their relatives of the first degree and in up to 1.5 percent of healthy controls. A positive correlation between the appearance of anti-Insulin and anti-islet cell antibodies has been reported.

Anti-Insulin autoantibodies may be detected several months and in some cases years before the onset of the fully clinical manifestation of the diseases. Occasionally also autoantibodies to Pro-Insulin may appear.

These "true" anti-Insulin autoantibodies directed against endogenous insulin have to be distinguished from those autoantibodies which are developed in insulin dependent diabetics undergoing therapy with insulin preparations of animal origin. In fact the latter have to be referred to side effects. These side effects may occur as local reactions of the skin by development of insulin-specific autoantibodies.
These autoantibodies are causing the formation of an insulin depot and they

may simulate a resistance against the hormonal treatment with animal insulin.

Additionally other immunological phenomenon have been reported for Type I diabetics. A lot of other autoantibody specificities have been detected in those patients, too, but these antibodies must not cause additional autoimmune phenomenon.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified mixture of bovine, porcine and recombinant human insulin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Insulin Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti-human IgG antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Insulin Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Insulin Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Concentration indicated on vial label)	6 vials (Ready-to-use)	4°C
Controls	2 vials (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 EDTA, heparin or citrate 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.

It is recommended to avoid grossly hemolyzed or lipemic samples.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled buffer before use.
- Patient sample: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, controls, and prediluted samples (1:100) into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl HRP-Antibody conjugate into each well. Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

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

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Negative: < 10 U/ml

Positive: ≥ 10 U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Insulin Ab ranged from 6.3-100 U/ml. The mean MDD was 0.5 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.1% and inter-assay precision was 5.2%.