Insulin Antibody ELISA Kit

Cat. No.:DEIA1829
Pkg.Size:96T

Intended use

The Kit is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against bovine, porcine and recombinant human insulin in human serum or plasma.

General Description

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 Langerhanns'sche 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 anti-bodies 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 Test

A mixture of highly purified preparations of bovine, porcine and recombinant human insulin is bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unbound serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG anti-bodies present in the original sample.

Reagents And Materials Provided

1. Divisible microplateconsisting of 12 modules of 8 wells each, coated with mixture of highly purified preparations of bovine, porcine and recombinant human insulin. Ready to use; 1 plate
2. Calibratorswith IgG class Anti-Insulin antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN3<0.1% (w/w)) containing: 0; 6.3; 12.5; 25;50; and 100 U/ml. Ready to use; 6 vials, 1.5 ml each 3. Anti-Insulin Controlsin a serum/buffer(PBS, BSA, NaN3<0.1% (w/w)) positive and negative , for the respective concentrations see the enclosed Quality Control Certificate. Ready to use; 2 vials, 1.5 ml each
4. Sample buffer(Tris, NaN3<0,1% (w/w)), yellow, concentrate (5x); 1 vial, 20 ml 5. Enzyme conjugatesolution (PBS, Proclin 300

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<0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use; 1 vial, 15 ml
6. TMB substrate solution. Ready to use; 1 vial, 15 ml
7. Stop solution(contains acid). Ready to use; 1 vial, 15 ml
8. Wash solution(PBS, NaN3<0.1% (w/w)), concentrate (50x); 1 vial, 20 ml

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm
2. Multi-Channel Dispenser or repeatable pipette for 100 µl
3. Vortex mixer
4. Pipettes for 10 µl, 100 µl and 1000 µl
5. Laboratory timing device
6. Data reduction software
7. Distilled or deionised water
8. Graduated cylinder for 100 and 1000 ml
9. Plastic container for storage of the wash solution

Storage

1. Store the kit at 2°C - 8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2°C - 8 °C.

Specimen Collection And Handling

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

Reagent Preparation

Sample Buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

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Controls are ready to use and need not be diluted.

Assay Steps

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and prediluted patient samples in duplicates.
2. Pipet 100 µlof calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µlof wash solution.
5. Dispense 100 µlof enzyme conjugate solution into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µlof wash solution.
8. Dispense 100 µlof TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature protected from light.
10. Add 100 µlof stop solution to each well of the modules and leave untouched for 5 minutes.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with reference at 600-650 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control and Negative Control as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfil-led, the results are invalid and the test should be repeated.

Calculation

For Anti-Insulin a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calibration

Since no international reference preparation for anti-Insulin autoantibodies is available, the assay system is calibrated in relative arbitrary units.

Typical Standard Curve

The figures below show typical results for Anti-Insulin ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

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No	Position	OD1	OD2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
STA	A 1/B 1	0.036	0.032	0.034	0.1	0.1	0.1	0.0	8
STB	C 1/D 1	0.354	0.345	0.349	6.3	6.1	6.2	6.3	2
STC	E 1/F 1	0.621	0.602	0.611	12.9	12.4	12.7	12.5	2
STD	G 1/H 1	0.984	1.005	0.994	25	25	25	25	1
STE	A 2/B 2	1.503	1.500	1.592	50	50	50	50	0
STF	C 2/D 2	2.57	2.035	2.046	102	99	100	100	1

Interpretation of Results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-Insulin test:

Anti-Insulin [U/ml]

normal: < 10

elevated: > 10

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges. The reference ranges below should be regarded as guidelines only.

Sensitivity

The lower detection limit for anti-Insulin has been determined at 0.5 U/ml.

Specificity

The microplate is coated with a mixture of highly purified preparations of bovine, porcine and recombinant human insulin. Therefore the anti-Insulin test kit recognizes only IgG-class auto-antibodies specific for these insulins. No cross reactivities have been observed.

Reproducibility

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay		
Sample No	Mean [U/ml]	CV [%]
1	11.2	2.5
2	27.6	2.9
3	59.7	4.0

Inter-Assay		
Sample No	Mean [U/ml]	CV [%]
1	11.6	6.0
2	31.2	5.2
3	69.5	4.3

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Insulin kit.

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Sample No	Dilution	Observed [U/ml]	Expected [U/ml]	Observed/ Expected
1	1:50	77.6		
	1:100	41.7	38.8	107%
	1:200	21.1	19.4	109%
	1.400	10.3	9.7	106%
	1:800	4.7	4.9	96%
2	1:100	100.7		
	1:200	50.7	50.4	101%
	1:400	23.7	25.2	94%
	1:800	11.1	12.6	88%
	1:1600	5.3	6.3	84%

Interferences

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants.
However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. Do not interchange kit components from different lots.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
4. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
5. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.)
6. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
10. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

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Limitations

The Anti-Insulin ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

Analyte Gene Information

Gene Name	INS insulin [Homo sapiens]
Official Symbol	INS
Synonyms	ILPR; IRDN; IDDM2; MODY10
GeneID	3630
mRNA Refseq	NM_000207.2
Protein Refseq	NP_000198.1
MIM	176730
UniProt ID	P01308
Chromosome Location	11p15.5
Pathway	ATF-2 transcription factor network; Adipogenesis; Aldosterone-regulated sodium reabsorption

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