

## Human IAA (Insulin Autoantibody) ELISA Kit

Cat. No.:DEIA2252

Pkg.Size:96T

### Intended use

This ELISA Kit is intended for measurement of Insulin Autoantibody in human serum.

### Principle Of The Test

Human insulin is immobilized onto microwells. The reference, high, and low controls, and diluted serum samples are added to the appropriate microwells. Human IgG specific antibodies to insulin in the serum sample and controls bind to the insulin molecules on the microwells. After washing off unreacted serum materials, an enzyme (alkaline phosphatase) labeled goat antibody specific to human IgG is added to the antigen antibody complex. After thorough washing to remove the unbound enzyme, a substrate (PNPP) solution is added and the color development is measured spectrophotometrically. The intensity of the color is directly proportional to the concentration of IAA in the sample. Two quality controls (high and low) are provided to monitor and validate assay results.

### Reagents And Materials Provided

1. IAA-Microwell Strips (with the holder): 12 strips
2. IAA-IgG Enzyme Conjugate (6X conc.): 2 x 1.0 mL
3. IAA-Sample Diluent (5X concentrate): 1 x 25.0 mL
4. Conjugate Diluent: 1 x 10.0 mL
5. IAA-Ref. Control (human serum): 1 x 1.5 mL
6. IAA-High Control (human serum): 1 x 1.5 mL
7. IAA-Low Control (human serum): 1 x 1.5 mL
8. Substrate Solution (PNPP): 1 x 15.0 mL
9. Washing Buffer (25 X): 1 x 20.0 mL
10. Stop Solution (1N NaOH): 1 x 6.0 mL

### Materials Required But Not Supplied

1. Distilled or deionized water.
2. Absorbent paper towels to blot dry the strips after washing and parafilm/plastic wraps to cover strips during incubations.
3. Suitable sized glass tubes for serum dilution.
4. Micropipet with disposable tips to deliver 10  $\mu$ L, 50  $\mu$ L and 100  $\mu$ L.
5. A microtiter plate washer or a squeeze bottle for washing.
6. 5 mL pipets for conjugate diluent delivery.
7. A 500 mL graduate cylinder.
8. Microtiter plate reader with 405 nm absorbance capability.
9. Plastic label tape, to tape unused wells before assay.

### Specimen Collection And Handling

Collect 5-10 mL of blood by venipuncture into a clot (red top) tube. Serum separators may be used. Separate serum by centrifugation. Serum samples may be stored at 2 °C - 8 °C.

Excessive hemolysis and the presence of large clots or microbial growth in the test specimen may interfere with the performance

of the test.

Freeze the serum sample at -20 °C if it cannot be analyzed within 24 hours.

## Reagent Preparation

### IAA-IgG Enzyme Conjugate Reconstitution:

Accurately transfer 5 mL of the Conjugate Diluent into one bottle containing IAA-IgG Enzyme Conjugate (concentrate).

Close the bottle and mix thoroughly by inversions.

Store the diluted conjugate at 2 °C - 8 °C at all times. Record the date of reconstitution on the label.

This diluted reagent expires 30 days after reconstitution.

Each of the two conjugate (concentrate) bottles is sufficient for 6 strips. Reconstitute as needed.

### IAA-Sample Diluent Buffer:

Transfer the entire contents (25 mL) into 100 mL of distilled/deionized water in a suitable container. Mix thoroughly;

label the container as IAA-Sample Diluent, and store at 2 °C - 8 °C.

The diluted reagent is stable until the expiration shown on the vial.

### Wash Solution:

Transfer the entire contents into 480 mL of distilled/deionized water in a 500 mL container. Mix thoroughly; label the container as wash solution, and store at 2 °C - 8 °C.

The diluted reagent is stable until the expiration shown on the vial.

### Serum Sample Preparation:

Accurately pipet 10 µL (0.010 mL) of serum sample into 1.0 mL of the Working Sample Diluent in an already labeled glass tube.

Mix thoroughly.

## Assay Steps

The test kit contains 12 microwell strips coated with human insulin. The number of microwell strips used in each assay depends upon the number of serum samples to be tested. If 12 microwell strips are used, a total 45 specimen sera can be tested in duplicate with this kit.

**IMPORTANT NOTE:** Bring all the reagents, including serum samples, to room temperature (25 °C) before starting the assay. Incubation temperatures varying by greater than  $\pm 1$  °C can definitely affect results.

1. Assemble the number of strips needed for a test run in the holder provided. The microwell strip must be snapped firmly in place or it may fall out and break.
2. Familiarize yourself with the indexing system of wells, e.g. well #A1, B1, C1, D1, etc. and label the strips used with a marking pen.
3. Dispense 100 µL of IAA-Reference Control into microwell C1 and D1
4. Dispense 100 µL of IAA-Low Control into microwells E1 and F1.
5. Dispense 100 µL of IAA-High Control into microwells G1 and H1.
6. Add 100 µL of diluted specimen serum (see #4, Section Reagent Preparation) to microwells A2 and B2. For more specimen samples, use additional strips and add diluted samples to microwells in duplicate. There should be 100 µL of solution in each microwell to be assayed except A1 and B1 which are empty at this point and will be used later.
7. Any wells not used on the strip should be properly covered and saved for the next run. Any well strips not used should be stored with the desiccant in the ziplock bag provided at 2 °C - 8 °C for the next run.
8. Cover the plate with a parafilm/plastic wrap (to prevent contamination) and leave at 2 °C - 8 °C overnight (12-16 hrs.).
9. The next morning, discard the solution into sink by quick decantation. Blot the plate dry by tapping gently on a paper towel. If an automatic plate washer is being used, wash each well 3 times with 300 µL of the Wash Solution (see #3, Section Reagent Preparation). If a squeeze bottle is used, fill the wells with Wash Solution carefully and decant the buffer from the microwells. Repeat the procedure two more times and blot the plate dry with a paper towel.
10. Add 100 µL of IAA-IgG Enzyme Conjugate reagent (see #1, section Reagent Preparation) to all microwells except wells A1

and B1.

11. Cover the plate with a parafilm/plastic wrap and let it stand at  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for one hour.
12. After incubation, repeat the washing step (step #9) and blot dry the microwells.
13. Add 0.1 mL (100  $\mu\text{L}$ ) of Substrate Solution to all microwells including wells A1 and B1. Be sure to dispense the substrate reagent at a rapid steady pace without any interruption.
14. Cover the plate and leave it in the dark for 30 minutes at room temperature ( $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ).
15. After 30 minutes promptly add 50  $\mu\text{L}$  of the Stopping Solution into each well at a rapid steady pace without any interruption.
16. Set up microplate reader to read the absorbance at 405 nm according to manufacturing instructions, and blank the plate reader with well A1 or B1.
17. Calculate the data according to Section 9.

## Quality Control

Low and High Controls must be run along with unknown samples each time in order for the results to be valid. The Low Control should show a ratio value  $< 0.95$  Units/mL and the High Control should show a value  $> 1.05$  Units/mL.

## Calculation

Record the spectrophotometric readings [optical density (OD) in absorbance units] as shown in the example IAA ELISA Sample Data. The actual OD reading from your I-IAA ELISA may be different. This is only an example.

1. Calculate the average O.D. reading of the Reference, Low and High Controls and specimen samples done in duplicate.  
Average OD (Mean OD) : Reference =  $R_m$ , Low =  $L_m$ , High =  $H_m$ , Samples =  $S_m$
2. Divide the average O.D. of Samples and Controls by the  $R_m$ -value. This gives a Ratio Value for each sample.

### IAA ELISA SAMPLE DATA

Section A: Control Results

Section B: Specimen Sample Results

Data (Net O.D.)			Ratio Value	Result
Controls	O.D.	Ave. O.D.		
Reference Ctrl	1.224 1.271	$R_m = 1.247$	1.00	
Low Ctrl	0.498 0.464	$L_m = 0.481$	0.39	Low
High Ctrl	1.855 1.764	$H_m = 1.809$	1.45	High

Data (Net O.D.)			Ratio Value	Result
Sample	O.D.	Ave. O.D.		
Reference Ctrl	1.224 1.271	$R_m = 1.247$	1.00	
1	1.994 2.010	$S1_m = 2.002$	1.61	High
2	0.540 0.542	$S2_m = 0.541$	0.43	Low
3	1.280 1.261	$S3_m = 1.270$	1.02	Indeterminate

## Precautions

1. The matrix of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because 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 as if potentially infectious.

2. Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.
3. Stopping Solution consists of 1N NaOH. This is a strong base and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.
4. Do not freeze test reagents, store all kit components at 2 °C - 8 °C at all times.
5. High and Low Controls must be run each time the test is performed.
6. Use only clear serum as test specimens. The test sample should not have gross turbidity, hemolysis, or microbial contamination.
7. All samples should be analyzed in duplicate.
8. Do not mix reagents from different lots.
9. Do not use expired reagents.
10. Do not allow reagents to stand at room temperature for extended periods of time.
11. Do not expose substrate solution to light.
12. Careful pipetting technique is necessary for reproducible and accurate results.

## Limitations

1. This is a qualitative test only.
2. Old substrate solution may give high background color.
3. Poor reproducibility may result from:
  - a. Inconsistent delivery.
  - b. Improper storage of reagents.
  - c. Improper reconstitution of reagents.
  - d. Faulty washing of microwells.
  - e. Inconsistent/defective instrument.
  - f. Using outdated reagents.

Therefore, it is very essential that the instructions are followed carefully and consistently. For better reproducibility, test conditions and test equipment should not vary extremely.

## REFERENCES

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