IAA ELISA

Catalog Number:
IAA31-K01

Enzyme immunoassay for the determination of **Autoantibodies** to **Insulin (IAA)** in human serum

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v. 1.0

LITERATURE

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PRINCIPLE of the TEST

IAA ELISA ASSAY KIT is an enzyme immunoassay for the quantitative determination of IgG autoantibodies and antibodies to insulin in human serum.

In the first step Insulin AAb from the diluted sample bind to human recombinant insulin coated on the microtiter plate. After an incubation of 60 minutes at 37 °C unbound components are removed by washing. In a next step bound antibodies reacts with the added anti-human-IgG horseradish peroxidase (HRP) complex. Excessive conjugate is removed after 15 minutes at 37 °C by another washing step. HRP converts the colorless substrate TMB added into a blue product. The enzyme reaction is stopped by adding an acid solution after 15 minutes at 37 °C. The absorbance of the resulting yellow product is measured at 450 / 620 nm within 30 minutes. The obtained OD is direct proportional to the amount of bound antibodies.

SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Do not use lipaemic or grossly hemolytic serum samples. Plasma should not be used.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, aliquot samples and keep them at - 20 °C.

TEST CONPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate** 12 breakable strips, 8 wells per strip coated with human recombinant insulin	vacuum sealed with desiccant
B WASHB	**Concentrated wash buffer** sufficient for 1000 ml	**100 ml** concentrate white capped
D CONJ	**Anti human IgG (sheep) Horser -peroxidase** (HRP) complex	**15 ml** ready for use red capped
E SUB	**Substrate** (3,3´,5,5´-Tetramethylbenzidin)	**15 ml** ready for use blue capped
F STOP	**Stop solution** (0.25 M sulfuric acid)	**15 ml** ready for use yellow capped
0 DIL	**Sample diluent**	**100 ml** ready for use black capped
C CONTROL	**positive control** concentration: see leaflet	**1 ml** ready for use red capped
1 - 5 CAL	**Calibrators:** concentrations see leaflet	**5 vials** **1 ml** each, ready for use white capped

Materials required

- Precision pipettes 5 - 1000 µl
- Multi-channel pipette
- Disposable pipette tips
- 8 channel wash comb or microplate washer
- Micro plate reader with optical filters for 450 nm and 620 or
- Graduated cylinders
- Distilled or de-ionized water
- Incubator 37 °C (can be purchased from MEDIPAN)
- Absorbent paper or paper towel
- foil

Size and storage

The IAA ELISA ASSAY KIT has been designed for 96 determinations. This is sufficient for the analysis of 42 unknown samples as well as for calibrators and control serum assayed in duplicates.

The expiry date of each component is reported on its respective label, that one of the complete IAA ELISA ASSAY KIT on the box label.

Upon receipt, all components of the IAA ELISA ASSAY KIT have to be kept at 2 - 8 °C, preferably in the original IAA ELISA ASSAY KIT box.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Samples have to be diluted 1 + 100
e.g. 5 µl sample + 500 µl sample diluent (0)

Please, handle the following components carefully:

A Allow the sealed microplate to reach room temperature before opening for at least 30 minutes. Unused wells should be stored refrigerated and protected from moisture in the original bag carefully resealed for 4 weeks.

B Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer (B) 1 + 9 with distilled or de-ionized water. For example, dilute 50 ml of the concentrate with 450 ml of distilled water. B should be free of crystals before dilution, otherwise dissolve by warming up to max. 37 °C. The diluted

washing solution can be stored at 2 - 8 °C up to 30 days.

D The anti-human IgG-HRP solution is stable up to 4 weeks at 2 - 8° C after opening.

E Avoid exposure of substrate solution (E) to light.

ASSAYS PROCEDURE

- Duplicates are recommended.

1. Pipette into the corresponding wells according to assay scheme
   - **100 µl** calibrators (1 - 5)
   - **100 µl** diluted sample and control serum (C).
2. Cover the plate and incubate for **60 min** at 37 °C.
3. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash **3 times** with **300 µl** washing solution (diluted from B) with 5 seconds soaking time each.
4. Add **100 µl** of anti-human IgG – HRP (D) to each well.
5. Cover the plate and incubate for **15 min** at 37 °C.
6. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash **3 times** with **300 µl** washing solution (diluted from B) with 5 seconds soaking time each.
7. Add **100 µl** substrate solution (**E**) to each well and shake shortly.
8. Incubate for **15 min** in the **dark** at 37 °C.
9. Add **100 µl** stop solution (**F**) to each well.

Avoid any time shift during pipetting the samples and reagents.

10. Read the optical density **at 450 nm** versus **620 or 690 nm** **within 30 min** after adding the stop solution.

Please note that the washing procedure is crucial. Insufficient washing will result to poor precision and falsely elevated OD readings. After each washing step any residual fluid has to be removed completely.
The plate should be shortly shaken after each pipetting step.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 5 on the ordinate, y-axis, versus their respective IAA-Ab-concentrations on the abscissa, x-axis.

The IAA Abs concentrations of the controls and the unknown diluted samples are directly read off in U/ml from the measured OD_{450} values. There is no further correction for the dilution necessary.

IAA ELISA ASSAY KIT may be used also with Computer Assisted Analysis with software able to use spline smoothing fitting.

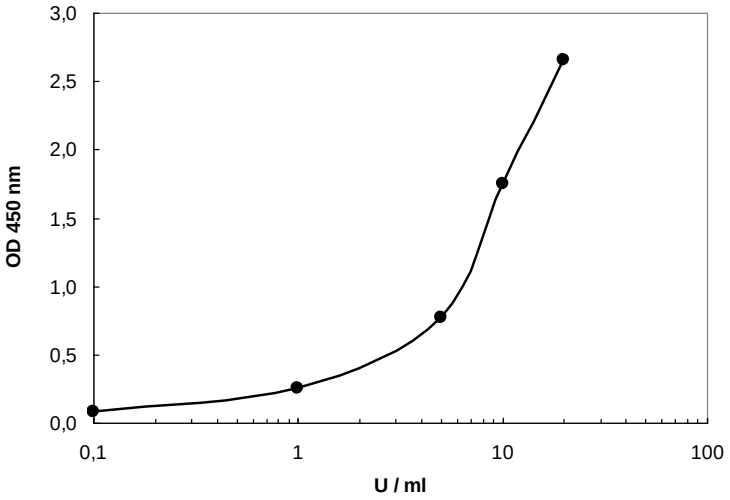
TYPICAL EXAMPLE

Do not use for evaluation!

Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	U / ml
Calibrator **1**	0.082	0.073	0.078	**0.1**
Calibrator **2**	0.288	0.226	0.257	**1**
Calibrator **3**	0824	0.720	0.772	**5**
Calibrator **4**	1.804	1.700	1.752	**10**
Calibrator **5**	2.697	2.607	2.652	**20**
Control **C**	1.360	1.323	1.342	8.0
Sample 1	0.540	0.530	0.535	3.0

STANDARD CURVE

Typical example



Criteria of validation

Specimens with an OD higher than Standard 5 should be diluted further by the sample diluent and the concentration of IAA / IA antibodies should be calculated by the applied dilution factor.

CHARACTERISTIC ASSAY DATA

Calibration

The IAA ELISA ASSAY KIT is artificially calibrated and concentrations of IAA are therefore expressed in U / ml.

Linearity

On the basic of the heterogeneous nature of the autoantibody population and in view of epitope specificity and affinity of the autoantibodies the theoretical values expected by dilution with IAA free human serum do not correspond with the measured concentrations in some cases.

Detection limits

The analytical sensitivity (lower detection limit, 0 + 3 SD) was established to be 0.08 U/ml.
The functional sensitivity was measured as 20 % of inter-assay CV at 0.9 U/ml.

Intra - and inter-assay variation

Intra-assay			Inter-assay		
Sample no.	Mean Concentration (U/ml)	CV (%)	Sample no.	Mean Concentration (U/ml)	CV (%)
1	1.8	9	4	2.4	8
2	6.0	7	5	8.2	6
3	14.3	9	6	17.2	10

IAA ELISA ASSAY KIT

ASSAY SCHEME

Bring all reagents to room temperature. Gently mix all reagents to ensure homogeneity.
Dilute all samples 1 + 100 (v + v) by sample diluent (0).

Step	Activity	Material	CAL	C	Diluted samples 1, 2 etc.
1	Pipette	Samples	100 µl	100 µl	100 µl
2	Incubate	Plate (A)	**1 hour at 37 °C**		
3	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution made from B	3 x 300 µl	3 x 300 µl	3 x 300 µl
4	Pipette	Anti-human IgG HRP (D)	100 µl	100 µl	100 µl
5	Incubate	Plate (A)	**15 min at 37 °C**		
6	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution made from B	3 x 300 µl	3 x 300 µl	3 x 300 µl
7	Pipette	Substrate (E)	100 µl	100 µl	100 µl
8	Incubate	Plate (A)	**15 min at 37 °C in the dark**		
9	Pipette and mix	Stop solution (F)	100 µl	100 µl	100 µl
10	Measure OD		at 450 nm versus 620 nm (or 690 nm) within 30 min		

SAFETY PRECAUTIONS

- **This kit is for in research use only**. Follow the working instructions carefully. This instruction manual is valid only for the present IAA ELISA ASSAY KIT with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/v) Thimerosal and (1 % v/v) Kathon as a preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.
- Since the IAA ELISA ASSAY KIT contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this IAA ELISA ASSAY KIT.

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.