EIASON® ICA

Enzyme immunoassay for the quantitative determination of both GAD_{65} and IA-2 autoantibodies in human serum

Instruction for use

For in-vitro use only

Product of

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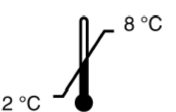


Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	BIOBU	Buffer for biotin
REF	Order number	CAL 0-6	Calibrators
	Product of	PC GAD PC IA-2	Positive control GAD_{65} Ab Positive control IA-2 Ab
2 °C 8 °C	Storage	WASH	Wash concentrate
CE	European Conformity	LOT	Batch code
	Expiry date	BIO	Biotin
PODBU	Diluent for SAPOD	SAPOD	Peroxidase
TURBO	Reaction enhancer	STOP	Stop solution
SUB	Substrate	MPL	Microplate

Intended use

For in-vitro use only.

The EIASON® ICA kit is an enzymeimmunoassay intended for the simultaneous quantitative determination of autoantibodies against GAD_{65} and IA2 in human serum. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of pancreas dysfunction.

Summary

Autoantibodies to pancreatic beta cell antigens are important serological markers of type 1 diabetes mellitus. The antigens recognised by these autoantibodies include insulin, glutamic acid decarboxylase (GAD_{65} kDa isoform) and an islet cell antigen (ICA) named IA-2 or ICA-512. The EIASON® ICA kit allows simultaneous measurement of GAD_{65} and IA-2 autoantibodies in the same sample.

Assay principle

In the EIASON® ICA kit, GAD_{65} and IA-2 autoantibodies (Ab) in patient sera, calibrators and controls are allowed to interact with GAD_{65} and IA-2 coated onto ELISA plate wells (1st incubation). The samples are then discarded, leaving any GAD_{65} or IA-2 autoantibodies in the patient sera, calibrators or controls bound to the GAD_{65} and IA-2 coated wells. A mixture of GAD_{65} Biotin and IA-2 Biotin is then added and during a second incubation step (through the ability of GAD_{65} and IA-2 autoantibodies to act divalently), a bridge is formed between the GAD_{65} or IA-2 bound to the wells and GAD_{65} Biotin or IA-2 Biotin respectively.
The amount of GAD_{65}/IA-2 Biotin bound is determined in a third incubation step by the addition of Streptavidin Peroxidase, which binds specifically to Biotin.
Excess unbound Streptavidin Peroxidase is then washed away and addition of 3,3',5,5' tetramethylbenzidine (TMB) results in formation of a blue colour. This reaction is stopped by addition of stop solution causing the well contents to turn from blue to yellow. The absorbance of the yellow reaction mixture at 450 nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of GAD_{65} or IA-2 autoantibody in the test sample. Reading at 405 nm allows quantitation of high absorbances.

Warnings and precautions

The EIASON® ICA kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Shelf life and storage of reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Storage and preparation of serum samples

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 100 µL is sufficient for one assay (duplicate 50 µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Do not use plasma in the assay. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 g in a microcentrifuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

Materials provided

Allow all reagents 1-11 to reach room temperature before use.

1. MPL ELISA strip wells coated with GAD_{65} and IA-2 (96 wells in total, 8 wells per strip). Before opening the packet of strip wells, allow it to stand at room temperature (20-25°C) for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided. Store at 2-8°C and use within 16 weeks. A frame for holding the wells during assays is also provided.
2. CAL 0-6 Calibrators; 0.7 mL each; 0, 4, 10, 20, 70, 145 and 450 u/mL; ready to use; corresponding to WHO reference preparation NIBSC 97/550
3. PC GAD GAD_{65} Ab and PC IA-2 IA-2 Ab Positive Controls; 0.7 mL each; ready to use
4. TURBO Reaction Enhancer; 4 mL; coloured red; ready to use
5. WASH Concentrated Wash Solution; 125 mL; 10 x concentrated; dilute 10 x with distilled water before use; store at 2 – 8°C up to kit expiry
6. BIOBU GAD_{65}/IA-2 Biotin reconstitution buffer for reconstituting BIO; 2 x 15 mL; coloured blue; ready to use
7. BIO GAD_{65}/IA2-biotin; 3 vials; lyophilized; reconstitute each vial with 5.5 mL of BIOBU; when more than one vial is used, pool the reconstituted vials and mix gently before use; use on day of reconstitution
8. PODBU Diluent for diluting SAPOD; 15 mL; ready to use
9. SAPOD Streptavidin-peroxidase; 0.7 mL; to be diluted 1 in 20 with PODBU (e.g. 0.5 mL + 9.5 mL); store at 2-8°C for up to 18 weeks after reconstitution
10. SUB Peroxidase substrate (tetramethyl benzidine); TMB; 15 mL; ready to use
11. STOP Stop solution; 12 mL; ready to use

Materials required but not provided in the kit

- ◊ Pipettes capable of dispensing 25 µL, 50 µL and 100 µL
- ◊ Means of measuring out various volumes to reconstitute or dilute reagents supplied
- ◊ Pure water
- ◊ ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm and 405 nm
- ◊ ELISA plate shaker, capable of 500 shakes/min (not an orbital shaker)
- ◊ ELISA plate cover

Assay procedure

General remarks

- ◊ Calculate the number of individual MPL wells needed for the assay. Allow all the reagents supplied including the appropriate number of strips to reach room temperature, fit the number of strip wells required firmly into the frame provided.
- ◊ All reagents must be mixed without foaming.
- ◊ Once the test has been started, all steps should be completed without interruption.
- ◊ Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross-contamination.
- ◊ Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- ◊ As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- ◊ Each run must include a standard curve and kit controls.

Assay procedure

1. Pipette 50 µL of CAL 0-6, PC GAD, PC GAD and test sera into the wells (in duplicate). Leave one well empty for blank.
2. Pipette 25 µL of TURBO except blank.
3. Cover the frame and shake the wells containing the various samples for 5 seconds (500 shakes per minute).
4. Incubate the plate overnight (16 – 20 h) at 2 – 8°C.
5. After the incubation discard the samples by briskly inverting the frame of strip wells over a suitable receptacle. Wash 3 times with 300 µL WASH per well and each time tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of wash buffer.
6. Pipette carefully 100 µL of reconstituted BIO into each well except blank.
7. Incubate for 1 hour at 18 - 22°C on an ELISA plate shaker (500 shakes per minute).
8. After the 1 hour discard the BIO by briskly inverting the wells over a suitable receptacle, wash 3 times with WASH as described under point 5.
9. Pipette carefully 100 µL of diluted SAPOD into each well except blank.
10. Incubate for 20 minutes at 20 - 25°C on an ELISA plate shaker (500 shakes per minute).
11. Discard the SAPOD by briskly inverting the wells over a suitable receptacle, wash 3 times with WASH as described under point 5.
12. Pipette carefully 100 µL of SUB into each well including blank.
13. Incubate for 20 minutes at 20 - 25°C in the dark during which time a blue colour will develop.
14. Stop the substrate reaction by careful addition of 100 µL of STOP to each well (this will cause the blue colour to turn yellow) and shake the plate for about 5 seconds on a plate shaker to ensure uniformity of the solution in each well. It is most important to ensure that the substrate incubation time (i.e. time from addition of SUB to addition of STOP) is the same for each well.
15. Measure the absorbance of each well at 450 nm and 405 nm for overrange filter (reference 620 – 650 nm) blanked against a well containing 100 µL of SUB and 100 µL of STOP within 10 minutes after adding the stop solution.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Calculation of results

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the absorbance of the calibrators on the y-axis (linear scale). The GAD_{65} and/or IA-2 autoantibody concentrations in patient sera can then be read off the calibration curve. Other data reduction methods can be used. Absorbance readings at 405nm can be converted to 450 nm absorbance values by multiplying by the appropriate factor (approximately 3.0 dependant on equipment being used). Values less than 25 u/mL should be read off a 450 nm curve.
Samples with high GAD_{65}Ab and IA-2Ab concentrations can be diluted in kit negative control CAL0 For example, 15 µL of sample plus 135 µL of negative control to give a 10 x dilution. Other dilutions (e.g. 100 x) can be prepared from a 10 x dilution or otherwise as appropriate. Some sera will not dilute in a linear way.

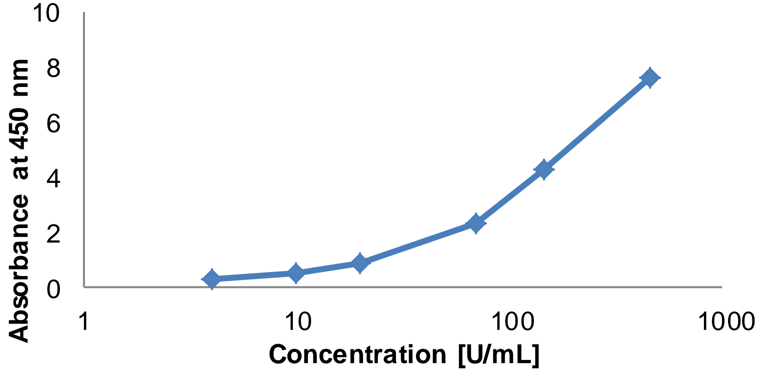
Sample assay data

Typical results obtained with EIASON® ICA calibrators:

Calibrator	Absorbance	
Units per mL	450 nm	405 nm
0	0.120	0.039
4	0.261	0.083
10	0.453	0.133
20	0.818	0.228
70	2.307	0.659
145	4.305	1.230
450	7.662	2.189

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



If results are to be expressed as an index, only the 4 u/mL calibrator CAL 0 need be included in the assay (all controls should still be included). The index values are calculated as follows:

Index = test sample absorbance at 450 nm ÷ CAL 0 absorbance

Healthy blood donor sera give index values of less than 1 suggesting that index values of 1 or more can be considered positive for GADAb and/or IA-2Ab.

Expected values

	NIBSC 97/550
	U/mL
Negative	<4
Positive	≥4

Each laboratory is recommended to determine ranges for their local population.

Quality control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC Laboratory are stated in the QC certificate added to the kit.
The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IASON directly.

Clinical evaluation

Clinical specificity and sensitivity

Sera from 70 healthy blood donors were all negative in the EIASON® ICA ELISA kit, although occasional healthy blood donors may have detectable GAD autoantibodies. Autoantibodies to GAD and/or IA2 were detected in 84% (n=216) of samples from patients with type 1 diabetes of various disease durations. In the DASP 2005 study, the EIASON® ICA showed 98% (n=100) specificity and 96% (n=50) sensitivity.

Lower detection limit: **0.17 U/mL**

(as the mean of 30 determinations of CAL 0 plus 2 standard deviations).

Precision

Intra-assay-precision (n=25)			Inter-assay-precision (n=28)		
Sample	Mean [U/mL]	CV [%]	Sample	Mean [U/mL]	CV [%]
1	6.6	6.3	1	115.2	3.4
2	25.7	4.7	2	21.2	4.4

Clinical accuracy

Analysis of sera from patients with autoimmune diseases other than type 1 DM indicated no interference from autoantibodies to the TSH receptor, thyroglobulin, thyroid peroxidase (TPO), ds-DNA, the acetylcholine receptor or from rheumatoid factor.

Interference

There are no interferences concerning the following substances:

- Intralipid up to 3000 mg/dl
- Bilirubin up to 20 mg/dl
- Haemoglobin up to 5 mg/mL

Legal aspects

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point Raliability of Resuts. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Useful publications

1. S. Chen et al. Sensitive non-isotopic assay for autoantibodies to IA2 and to a combination of both IA2 and GAD_{65}. Clinica Chimica Acta 2005 357: 74-83
2. C. Törn et al. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008 51:846-852.

Pipetting scheme

Allow all reagents to reach room temperature before use

Step			
1. Pipetting (Except blank)	CAL 0-6 **50 µL**	PC GAD PC IA-2 **50 µL**	Samples **50 µL**
2. Pipetting	TURBO **25 µL** except blank		
3. Shaking	Shaking 5 sec. on a shaker (500 shakes/min)		
4. Incubation	16-20 hours at 2 - 8°C		
5. Washing	wash 3x : aspirate or decant add **300µL** WASH aspirate or decant and dry on an absorbent material		
6. Pipetting	BIO **100µL** except blank		
7. Incubation	1 hour on a shaker (500 shakes/min) (18 -22°C)		
8. Washing	wash 3x : see step 5		
9. Pipetting	SAPOD **100µL** except blank		
10. Incubation	20 minutes at room temperature on a shaker (500 shakes/min; 20-25°C)		
11. Washing	wash 3x: see step 5		
12. Pipetting	SUB **100µL** including blank		
13. Incubation	20 minutes at room temperature in the dark (20 - 25°C)		
14. Pipetting	STOP **100µL** including blank		
15. Shaking	5 sec. on a shaker		
16. Reading	450nm (RF 620nm) Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 10 min. Calculation: 4-parameter or point to point		

Expected values

	NIBSC 97/550
	U/mL
Negative	<4
Positive	>4