2 Screen Islet Cell Autoantibody Human ELISA

**Cat. No.:DEIA4453**
**Pkg. Size: 96T**

### Intended use

The 2 Screen Islet Cell autoantibody (2 Screen) ELISA kit is intended for use by professional persons only, for quantitative determination of both GAD and IA-2 autoantibodies in human serum.

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 (GAD65 kDa isoform) and an islet cell antigen named IA-2 or ICA-512. The 2 Screen ELISA allows simultaneous measurement of GAD and IA-2 autoantibodies in the same sample.

### General Description

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 (GAD65 kDa isoform) and an islet cell antigen named IA-2 or ICA-512. 2 Screen ELISA allows simultaneous measurement of GAD and IA-2 autoantibodies in the same sample.

### Principle Of The Test

In 2 Screen ELISA, GAD and IA-2 autoantibodies (Ab) in patient sera, calibrators and controls are allowed to interact with GAD65 and IA-2 coated onto ELISA plate wells (1st incubation). The samples are then discarded, leaving any GAD or IA-2 autoantibodies in the patient sera, calibrators or controls bound to the GAD65 and IA-2 coated wells. A mixture of GAD Biotin and IA-2 Biotin is then added and during a second incubation step (through the ability of GAD and IA-2 autoantibodies to act divalent), a bridge is formed between the GAD65 or IA-2 bound to the wells and GAD Biotin or IA-2 Biotin respectively. The amount of GAD/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 450nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of GAD or IA-2 autoantibody in the test sample. Reading at 405nm allows quantitation of high absorbances.

### Materials Required But Not Supplied

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

### Storage

Store the kit at 2–8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

### Specimen Collection And Handling
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 microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

**Reagent Preparation**

**DAY ONE:**
- **A:** GAD65 and IA-2 Coated Wells, 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag.
  - Ensure stripwells are firmly fitted into frame provided. After opening return any unused wells to the original foil packet with desiccant provided and seal with adhesive tape. Place foil bag in the self-seal plastic bag and store at 2-8°C for up to 16 weeks.
- **B:** Reaction Enhancer
  - 4 mL Coloured red
  - Ready for use
- **C1-6:** Calibrators
  - 4, 10, 20, 70, 145 and 450 u/mL (units are NIBSC 97/550)
  - 6 x 0.7 mL
  - Ready for use
- **D1:** GAD Ab Positive control
  - 0.7 mL
  - Ready for use
- **D2:** IA-2 Ab Positive Control
  - 0.7 mL
  - Ready for use
- **D3:** Negative Control
  - 0.7 mL
  - Ready for use

**DAY TWO:**
- **E:** GAD/IA-2 Biotin (GAD Biotin plus IA-2 Biotin)
  - 3 vials lyophilised
  - Reconstitute each vial with the amount of GAD/IA-2 Biotin reconstitution buffer (F) shown on the vial label. When more than one vial is used, pool the reconstituted vials and mix gently before use. Use on day of reconstitution.
- **F:** GAD/IA-2 Biotin reconstitution buffer
  - 2 x 15 mL Coloured blue
  - Ready for use
- **G:** Streptavidin Peroxidase (SAPOD)
  - 1 x 0.7 mL
  - Concentrated
  - Dilute 1 in 20 with diluent for SAPOD (H). For example, 0.5 mL (G) + 9.5 mL (H).
  - Store at 2 – 8°C for up to 18 weeks after dilution.
- **H:** Peroxidase Substrate (TMB)
  - 15 mL
  - Ready for use
Ready for use
Concentrated wash solution
125 mL
J: Concentrated
Dilute 10 X with pure water before use. Store at 2 – 8°C up to kit expiry.
Stop solution
K: 12 mL
Ready for use

Assay Steps

Allow all reagents to stand at room temperature for at least 30 minutes before use. A repeating Eppendorf type pipette is recommended for steps 2, 6, 9, 11 and 12.

1. Pipette 50 µL of patient sera, calibrators (C1-6) and controls (D1, D2 and D3) into respective wells in duplicate, leaving one well empty for blank (see step 13).
2. Pipette 25 µL of reaction enhancer (B) into each well (except blank).
3. Cover the frame and shake the wells for 5 seconds on an ELISA plate shaker (500 shakes per min).
4. Incubate the plate at 2 – 8°C (without shaking) overnight (16-20 hours)
5. After this overnight incubation, aspirate the samples and wash the plate 3 times with wash solution (J) using a plate washer. (If a plate washer is not available, discard the samples by briskly inverting the frame of stripwells over a suitable receptacle, wash the wells 3 times manually and after the final wash invert the frame of wells and tap gently on a clean dry absorbent surface to remove excess wash solution).
6. Pipette 100 µL of reconstituted GAD/IA-2 Biotin (E) into each well (except blank). Avoid splashing the material out of the wells during addition.
7. Cover the plate, and incubate at 18 - 22 ºC for 1 hour on an ELISA plate shaker (500 shakes per min).
8. Repeat wash step 5.
9. Pipette 100 µL of diluted streptavidin peroxidase (G) into each well (except blank) and incubate at room temperature for 20 minutes, on an ELISA plate shaker (500 shakes per min).
10. After the incubation, wash the wells three times with diluted wash solution (J) as in step 5 (in the case of washing manually, use an additional final wash step with pure water to remove any foam).
11. Pipette 100 µL of TMB (I) into each well (including blank) and incubate in the dark at room temperature for 20 minutes without shaking.
12. Pipette 100 µL stop solution (K) into each well (including blank) and shake the plate for approximately 5 seconds on a plate shaker (500 shakes per min). Ensure substrate incubations are the same for each well.
13. Read the absorbance of each well at 405 nm and then 450 nm using an ELISA plate reader, blanked against a well containing 100 µL of TMB substrate (I) and 100 µL Stop solution (K) only. This step should be completed within 10 minutes of adding the stop solution (step 12).

Calculation

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 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 3.5, dependant on equipment being used). Values less than 25 u/mL should be read off a 450 nm curve.

Samples with high GADAAb and IA-2Ab concentrations can be diluted in kit negative control (D3). For example, 15 L of sample plus 135 L of negative control to give a 10x dilution. Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate. Some sera will not dilute in a linear way.
**Index Calculation**

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

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\text{Index} = \frac{\text{test sample absorbance at 450nm}}{4 \text{ u/mL calibrator absorbance at 450nm}}
\]

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.

**Typical Standard Curve**

Example only; not to be used for calculation of actual results

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**Interpretation of Results**

Negative < 4 u/mL
Positive ≥ 4.0 u/mL

**Sensitivity**

0.17 U/ml

**Specificity**

Sera from 70 healthy blood donors were all negative in the 2 Screen ELISA, 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 2 Screen ELISA showed 98% (n=100) specificity.

**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, ds-DNA the acetylcholine receptor or from rheumatoid factor.
Reproducibility

Intra-Assay Precision, CV%: 4.7-6.3
Inter-Assay Precision, CV%: 3.4-4.4

Interferences

No interference was observed when samples were spiked with the following materials: haemoglobin up to 5mg/mL, bilirubin up to 20 mg/dL or intralipid up to 3000 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for GAD and/or IA-2 autoantibody levels with the assay.

REFERENCES

1. S. Chen et al. Sensitive non-isotopic assays for autoantibodies to IA2 and to a combination of both IA2 and GAD65. Clinica Chimica Acta 2005 357: 74-83