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DCM084-7
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IA2

for routine analysis

Quantitative determination of autoantibodies to "Protein Tyrosine Phosphatase IA2" in human serum or plasma

RUO | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO084

INTENDED USE

IA2 ELISA kit is an enzyme immunoassay for the quantitative determination of autoantibodies to Protein Tyrosine Phosphatase (IA2 Abs) in human serum or plasma.
IA2 kit is intended for research use only.

1. CLINICAL SIGNIFICANCE

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to an environmental agent. Autoimmune destruction of beta cells is thought to be completely asymptomatic until 80 - 90% of the cells are lost. This process may take years to complete and may occur at any time. During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies are present years before the onset of type 1 diabetes and prior to clinical symptoms. Early studies utilized the immunofluorescence test for islet-cell antibodies (ICA), which has been difficult to Calibratorize and is now replaced by a combination of several radioimmunoassays for antibodies against specific beta cell antigens, such as insulin (IAA), glutamic acid decarboxylase (GAD) and tyrosine phosphatase ICA 512 (IA2). IA2, a member of the protein tyrosine phosphatases family is localized in the dense granules of pancreatic beta cells and the second defined recombinant islet cell antigen. IA2 shares sequence identity with the islet cell antigen 512. The higher frequency of antibodies to IA2 is explained by the presence of autoantibodies directed to the COOH terminus of IA2 which is lacking in the ICA512 molecule. IA2 autoantibodies are present in the majority of individuals with new-onset type 1 diabetes and in individuals in the pre-diabetic phase of the disease. The appearance of autoantibodies to IA2 seems to be correlated with the rapid progression to overt type 1 diabetes.
The combination of tests for GAD65 and IA2 autoantibodies is highly relevant for risk assessment of type 1 diabetes in children and adolescence. The screening for GAD65 and IA2 autoantibodies detect more than 90 % of subjects at risk for type 1 diabetes and may, therefore, possess the potential to replace ICA technique.

2. PRINCIPLE

The assay system uses the ability of IA2 antibodies to act divalently and form a bridge between immobilized IA2 and liquid-phase IA2-Biotin. In the first step IA2 antibodies from the sample bind to IA2 coated on the microtiter plate. In a second step IA2-Biotin binds to this complex. The bound IA2-Biotin correlates with the amount of IA2 antibodies in patient's serum. Unbound IA2-Biotin is removed by the washing step.
The bound IA2-Biotin is quantified by addition of Streptavidin-peroxidase and a chromogenic substrate (TMB) and reading the optical density (OD) at 450 nm. The concentration of anti IA2 antibodies is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (4 vials, 0.7 mL each)

CAL1	REF DCE002/8407-0
CAL2	REF DCE002/8408-0
CAL3	REF DCE002/8409-0
CAL4	REF DCE002/8410-0

2. Controls (2 vials, 0.7 mL each, ready to use)

Negative Control	REF DCE045/8401-0
Positive Control	REF DCE045/8402-0

3. Enhancer (1 vial, 4 mL) REF DCE052-0
4. Streptavidin-peroxydase (1 vial, 0.7 mL) REF DCE041/8441-0
5. Streptavidin-peroxydase diluent (1 vial, 15 mL) REF DCE048/8448-0
6. Biotin (3 vial, lyophilised) REF DCE019/8419-0
7. Biotin diluent (2 vials, 15 mL each) REF DCE047/8447-0
8. Coated Microplate (1 breakable microplate)
Human recombinant IA2 adsorbed on the microplate REF DCE002/8403-0
9. Substrate solution (1 vial, 15 mL) REF DCE004/8404-0
10. Stop solution (1 vial, 12 mL)
0.25M sulphuric acid REF DCE005/8405-0
11. 10X Conc. Wash Solution (1 vial, 125 mL) REF DCE006/8406-0

3.2. Necessary reagents not supplied

Distilled or deionized water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm, 620-630 nm).

Notes

Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 8 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable 1 month at 2-8°C.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, Calibrators and Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or ProClin™ 300 as preservative. Avoid contact with skin or mucosa.
- Classification according to Regulation (EC) No. 1272/2008 [CLP]

Streptavidin-peroxydase
Skin sensitivity, Category 1

Warning

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements:
P261 - Avoid breathing dust / fume / gas / mist / vapours / spray.
P272 - Contaminated work clothing should not be allowed out of the workplace
P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
P302+P352 - IF ON SKIN: Wash with plenty of soap and water
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Substrate solution
Reproductive toxicity, Category 1B

Danger

Hazard statements:
H360D - May damage the unborn child.

Precautionary statements:
P202 - Do not handle until all safety precautions have been read and understood.
P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
P308+P313 - IF exposed or concerned: Get medical advice/attention.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Enhancer
Skin sensitivity, Category 1

Warning

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements:
P261 - Avoid breathing mist, vapours.
P272 - Contaminated work clothing should not be allowed out of the workplace
P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
P302+P352 - IF ON SKIN: Wash with plenty of soap and water
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Streptavidin-peroxydase diluent
Hazard statements:
EUH208 - May produce an allergic skin reaction.

Precautionary statements:
N/A

- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Biotin

Prepare the IA2-Biotin solution by reconstitution of one vial of lyophilized IA2-Biotin with X mL diluent for IA2-Biotin directly prior to use. The amount X for reconstitution is shown on the certificate of quality enclosed.
Reconsitute and use immediately before using and within the day.

6.2. Preparation of the Wash Solution

Prepare a sufficient amount of washing solution by diluting the 10X Conc. Wash Solution 1:10 with distilled or deionized water. For example, dilute 50 mL of the Concentrate Wash with 450 mL of distilled water. The solution 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.

6.3. Preparation of the Streptavidin-peroxydase

Prepare a sufficient amount of Streptavidin-peroxidase solution by diluting the concentrated streptavidin-peroxydase 1:20 with diluent (0.25 mL streptavidin concentrate with 4.75 mL diluent for streptavidin-peroxydase). The solution prepared is stable up to 4 weeks at 2-8°C.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the

calibration curve (C_1-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Sample or Controls		**50 µL**	
Calibrator C_1-C_4	**50 µL**		
Enhancer	**25 µL**	**25 µL**	
Cover the plate, shake > 500 rpm for 5s. Incubate at 2-8°C overnight (at least 16h). Allow covered plate to reach room temperature (22- 28°C). Remove the content from each well and wash the wells 3 times with 300 µL of diluted Wash Solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.			
Biotin	**100 µL**	**100 µL**	
Cover the plate and incubate at 2-8°C for 1 hour without shaking. Remove the content from each well and wash the wells 3 times with 300 µL of diluted Wash Solution. **Washing**: follow the same indications of the previous point.			
Streptavidin peroxydase	**100 µL**	**100 µL**	
Cover the plate (to prevent contamination).Incubate at room temperature (22-28°C) for 20 minutes while shaking > 500 rpm. Remove the content from each well and wash the wells 3 times with 300 µL of diluted Wash Solution. **Washing**: follow the same indications of the previous point.			
Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22-28°C) for 20 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the plate for 5 second > 200 rpm. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

Please note that the washing procedure is crucial. Insufficient washing will result to poor precision and falsely elevated OD readings. Without shaking the OD will be 20% lower with a conseguent loss of sensitivity.

7. RESULTS

7.1. Calibration curve

The calibration curve is established by plotting the mean OD-values of the Calibrators 1-4 on the ordinate, y-axis, versus their respective IA2 Ab-concentrations on the abscissa, x-axis. In addition the negative control (CI) should be used (see below).
The IA2 Abs concentrations of the controls and the unknown samples are directly read off in IU/mL from the measured OD450 values.
The IA2 kit may be used also with Computer Assisted Analysis using software able to curves with spline smoothing fit.
Example:

Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	IU/mL
Neg Control	0.076	0.078	0.077	0
Calibrator 1	0.226	0.230	0.228	7.5
Calibrator 2	0.633	0.662	0.648	35
Calibrator 3	2.672	2.835	2.754	120
Calibrator 4	3.642	3.744	3.693	350
Pos Control	---	---	---	---
Patient 1	0.517	0.547	0.532	43.6

7.2. Reference values

IA2	
Negative	< 7.5 IU/mL
Positive	≥ 7.5 IU/mL

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

8. PERFORMANCE AND CHARACTERISTICS

8.1. Calibration

IA2 kit is calibrated against the WHO reference preparation NIBSC 97/550 and concentrations of IA2 Abs are therefore expressed in IU/mL.

8.2. Linearity

On the basic of the heterogeneous nature of the autoantibody population and in view of epitope specificity and affinity of the autoantibodies exceptions are possible in some cases.

8.3. Specificity and Sensitivity

Using a cut-off of 7.5 IU/mL, IA2 kit shows a sensitivity of 65.3% and specificity of 100%, regarding patients with newly onset type 1 diabetes.

8.4. Detection Limits

The analytical sensitivity of IA2 kit was established to be 0.37 IU/mL.

8.5. Intra and inter-assay variations

8.5.1. Intra-Assay

Within run variation was determined by replicate 12 times three different sera with values in the range of

calibration curve. The within assay variability is ≤ 4.6%

8.5.2. *Inter-Assay*

Between run variation was determined by replicate the measurements of one control serum with different lots of kits and/or different mix of lots of reagents. The between assay variability is ≤ 4.5%.

9. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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