INSTRUCTION MANUAL

(April 01, 2014)

Medizym® anti-IA2

- 96 determinations -

REF **3803**

Enzyme immunoassay for the determination of **autoantibodies** to **Protein Tyrosine Phosphatase IA2** in human serum

MEDIPAN GMBH

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IFU symbols non-radioactive assays MEDIPAN GMBH

RUO	For Research Use Only	LOT	Batch code
REF	Catalogue number		Manufactured by
	Expiry date		Consult operating instruction
	Consult accompanying documents		Biological risk
	Store at	WASHB	Wash buffer
MP	Microtiter plate	SUB	Substrate
CONJ	Conjugate	BUF D	Diluent for conjugate
STOP	Stop solution	BUF H	Diluent for start buffer
START	Start buffer	CAL	Calibrators
CONTROL	Control serum	ENH	Enhancer

Manufactured under license to patents including EP 1448993 B1

INTENDED USE

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 standardize and is now replaced by a combination of several radioimmunoassays for antibodies against specific beta cell antigens, such is 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.

LITERATURE

- Lan MS, Wasserfall C, Maclaren NK & Notkins AL: IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus; Proc. Natl. Acad. Sci. USA 1996, 93: 6367-6370
- Pietropaolo M, Hutton JC & Eisenbarth GS: Protein tyrosine phosphatase-like proteins: Link with IDDM; Diabetes Care 1997, 20: 208-214
- Batstra M, HJ Anstoot & P Herbrink: Prediction and diagnosis of type 1 diabetes using ß-cell autoantibodies; Clin Lab 2001, 47: 497-507
- Seissler J, E.Hatziagelaki & WA Scherbaum: Modern concepts for the prediction of type 1 diabetes; Exp Clin Endocrinol Diabetes 2001, 109 Suppl 2: S304-S316
- Pozzilli P, S Manfrini & L Monetini: Biochemical markers of type 1 diabetes; clinival use, Scand J Clin Lab Invest 2001, 61: 38-44
- Winter WE, N Harris & D Schatz: Immunological markers in the diagnosis and prediction of autoimmune Type 1a diabetes; Clinical Diabetes 2002, 20: 183-191

PRINCIPLE of the TEST

Medizym® anti-IA2 is an enzyme immunoassay for the quantitative determination of autoantibodies to Protein Tyrosine Phosphatase (IA2 Abs) in human serum.
The assay system uses the ability of IA2 Abs acting divalently and forming a bridge between immobilized IA2 and liquid-phase IA2-Biotin. In the first step IA2 Ab 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 Abs in patient's serum. Unbound IA2-Biotin is removed by washing.
The bound IA2-Biotin could be quantified by addition of Streptavidin-peroxidase and a colorogenic substrate (TMB) and reading the optical density (OD) at 450 nm.

SAMPLE PREPARATION

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Should be avoided use lipemic or grossly hemolytic serum samples and plasma.

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, initially aliquot samples and keep at - 20 °C.

TEST COMPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate** 12 breakable strips per 8 wells coated with human recombinant IA2		vacuum sealed with desiccant
B WASHB	**Concentrated wash buffer** sufficient for 1250 ml		**125 ml** concentrate
D CONJ	**Streptavidin-peroxidase** (SA-POD) sufficient for 14.0 ml		**0.7 ml** concentrate
E SUB	**Substrate** (3,3´,5,5´-Tetramethylbenzidin)		**15 ml** ready for use
F STOP	**Stop solution** (0.25 M sulfuric acid)		**12 ml** ready for use
G BUF D	**Diluent for SA-POD (D)**		**15 ml** ready for use
H START	**IA2-Biotin**		**3 vials** lyophilized
J BUF H	**Diluent for IA2-Biotin (H)**		**2 x 15 ml** ready for use colored blue
K ENH	**Enhancer**		**4 ml** ready for use colored red
C I CONTROL	**negative control**	L	**0.7 ml** ready for use
C II CONTROL	**positive control** concentration: see leaflet	L	**0.7 ml** ready for use
1 - 4 CAL	**calibrators** concentration: see leaflet	L	**4 vials** 0.7 ml each, ready for use

Materials required

- Precision pipettes 10 - 100 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 690 nm
- Graduated cylinders
- Distilled or de-ionized water
- Absorbent paper or paper towel
- foil

Size and storage

Medizym® anti-IA2 has been designed for 96 determinations. This is sufficient for the analysis of 42 unknown samples as well as for blank, calibrators and control sera assayed in duplicates.
The expiry date of each component is reported on its respective label, that of the complete kit on the box label.
Upon receipt, all components of the Medizym® anti-IA2 have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow samples and all test components to reach room temperature prior to assay (at least 30 minutes). Take care to agitate serum samples gently in order to ensure homogeneity.

Please, handle carefully with the following components:

A Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original bag carefully resealed. Use within 16 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 Prepare a sufficient amount of Streptavidin-peroxidase solution by diluting SA-POD concentrate (D) 1 + 19 (0.25 ml SA-POD concentrate with 4.75 ml diluent for SA-POD (G)**.** The SA-POD solution prepared is stable up to 16 weeks at 2 - 8 °C.

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

H Prepare the IA2-Biotin solution by reconstitution of one vial lyophilized IA2-Biotin (H) with **x** ml diluent for IA2-Biotin (J) directly prior to use. The amount **x** for reconstitution is shown on the leaflet enclosed. Use within the day of reconstitution.

ASSAYS PROCEDURE

- Duplicates are recommended.

1. Pipette into the corresponding wells according to assay scheme
   - **50 µl** negative control (C I) and calibrators (1 - 4)
   - **50 µl** samples or control serum (C II).
2. Pipette **25 µl** Enhancer (K) into each well.
3. Cover the plate, shake for 5 seconds > 500 rpm and incubate **over night** (at least 16 h) at 2 - 8 °C.
4. Prepare sufficient amount of reagents (B, D / G, E, H/J) and allow the covered plate to reach **room temperature** (at least 30 minutes preferable while shaking – possible precipitates will disappear).
5. 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.
6. Add **100 µl** of reconstituted IA2-Biotin solution (prepared from H and J) to each well.
7. Cover the plate and incubate for **60 min** at room temperature (18 - 25 °C) while shaking > 500 rpm.
8. 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.
9. Add **100 µl** diluted SA-POD (prepared from D and G) to each well.
10. Cover the plate and incubate for **20 min** at room temperature (18 - 25 °C) while shaking > 500 rpm.
11. 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.
12. Add **100 µl** substrate solution (E) to each well and shake for 5 seconds.
13. Incubate for **20 min** in the **dark** at room temperature.
14. Add **100 µl** stop solution (F) after exact 20 min for each well. Shake the plates for 5 seconds > 200 rpm.
15. Read the optical density **at 450 nm** versus 620 nm (690 nm) within **5 minutes** 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.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the standards 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 OD_{450} values.
High concentrations of IA2 Abs could be measured by reading absorbencies at 405 nm instead of 450 nm.

Medizym® anti-IA2 may be used also with Computer Assisted Analysis using software able to curves with spline smoothing fit.

TYPICAL EXAMPLE

Do not use for evaluation!

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Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	IU / ml
Control **CI**	0.076	0.078	0.077	**1**
Calibrator **1**	0.226	0.230	0.228	**15**
Calibrator **2**	0.633	0.662	0.648	**60**
Calibrator **3**	2.672	2,835	2.754	**200**
Calibrator **4**	3.642	3.744	3.693	**400**
Control **CII**				
Sample 1	0.517	0.547	0.532	43.6

STANDARD CURVE

Typical example

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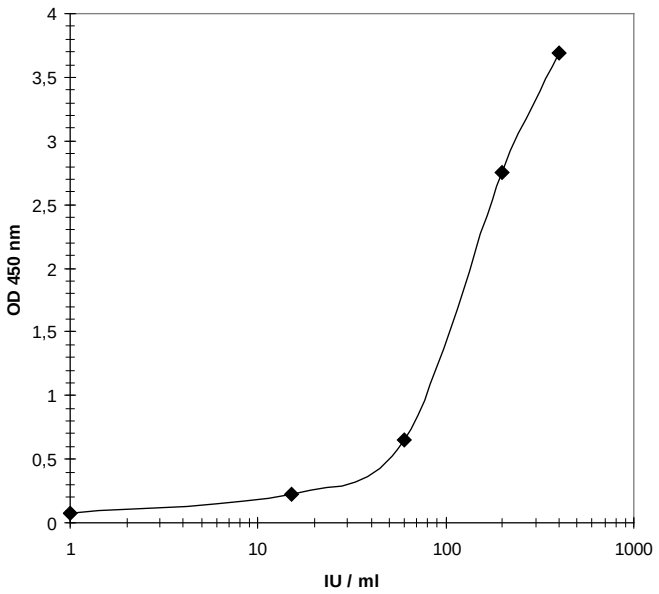

REFERENCE VALUES

Medizym® anti-IA2	
negative	< 8 IU/ml
grey zone	8 - 10 IU/ml
positive	≥ 10 IU/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-IA2 antibodies levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide only a guide.

CHARACTERISTIC ASSAY DATA

Calibration

The Medizym® anti-IA2 is calibrated against the WHO reference preparation NIBSC 97/550 and concentrations of IA2 Abs are therefore expressed in IU/ml.

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.

Specificity and sensitivity

Using a cut-off of 10 IU/ml the Medizym® anti-IA2 shows a sensitivity of 75 % and specificity of 98 %, regarding type 1 diabetes.

Detection limits

The analytical sensitivity (lower detection limit, 0 ± 3 SD) was established to be 0.5 IU/ml.
The functional sensitivity was measured as 20 % of inter-assay CV at 0.8 IU/ml.

Intra - and inter-assay variation

Intra-assay			Inter-assay		
Sample no.	Mean Concentration (IU/ml)	CV (%)	Sample no.	Mean Concentration (IU/ml)	CV (%)
1	24	4	5	1	6
2	48	4	6	14	7
3	118	2	7	136	9
4	259	5	8	182	8

LIMITATIONS of the METHOD

Healthy individuals should be tested negative by using the Medizym® anti-IA2. However, IA2 Abs may also be present in apparently healthy persons.

Medizym® anti-IA2

Bring all reagents to room temperature. Gently mix all reagents to ensure homogeneity.

ASSAY SCHEME

Step	Activity	Material	CI / CAL	Control serum (C II)	Sample 1, 2 etc.
1	Pipette	Samples	50 µl	50 µl	50 µl
2	Pipette	Enhancer	25 µl	25 µl	25 µl
3	Incubate	Plate	Incubate at 2 - 8 °C **for at least 16 h**		
4	Prepare	Reagents B, D/G, E, H/J and sealed plate	bring to **room temperature** (plate preferable while shaking for at least 30 minutes)		
5	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution	3 x 300 µl	3 x 300 µl	3 x 300 µl
6	Pipette	IA2-Biotin solution	100 µl	100 µl	100 µl
7	Incubate	Plate	**1 hour** at room temperature **with shaking (> 500 rpm)**		
8	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution	3 x 300 µl	3 x 300 µl	3 x 300 µl
9	Pipette	SA-POD solution	100 µl	100 µl	100 µl
10	Cover and incubate	Plate	**20 min** at room temperature **with shaking (> 500 rpm)**		
11	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution	3 x 300 µl	3 x 300 µl	3 x 300 µl
12	Pipette	Substrate	100 µl	100 µl	100 µl
13	Incubate	Plate	**20 min** at room temperature **in the dark**		
14	Pipette and mix	Stop solution	100 µl	100 µl	100 µl
15	Measure OD		at 450 nm against 620 nm (690 nm) within 5 min		

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. This instruction manual is valid only for the present 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) of sodium azide as a preservative. 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 patient samples as if potentially hazardous.
- Since the 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 kit.