Instructions For Use

Valid as of 13.03.2023

Medizym® anti-GAD M

REF 3507

Enzyme immunoassay for the determination of antibodies against Glutamic Acid Decarboxylase (GAD_{65}) in human serum

distributed in the US/Canada by:
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Σ 96

2°C – 8°C

1 Intended Purpose

The Medizym® anti-GAD M is a quantitative immunoassay for the determination of antibodies against Glutamic Acid Decarboxylase (GAD_{65}) in human serum.

The Medizym® anti-GAD M is intended as an aid in the diagnosis of diabetes mellitus type 1 in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual professional *in vitro* diagnostic use.

2 Diagnostic Relevance

Diabetes mellitus type 1 is a chronic autoimmune disease in which the insulin-producing beta cells of the islets of Langerhans in the pancreas are destroyed. The consequence of this destruction is a reduced insulin production, which results in high blood sugar levels as diabetes mellitus. Genetic predispositions and viral infections are considered risk factors, but the exact causes have not yet been fully clarified.

The destruction of the insulin-producing beta cells of the pancreas is based on the presence of islet cell antibodies (ICA), which are directed against different antigens of the pancreatic islet cells, such as glutamic acid decarboxylase (GAD65), tyrosine phosphatase (insulinoma-associated antigen 2, IA2), the zinc transporter 8 (ZnT8) and against insulin. Islet cell antibodies (ICA) can be detected in 70 – 80 % of patients with diabetes mellitus. The different antibodies usually appear months to years before the occurrence of elevated blood sugar levels and are therefore also considered important prognostic markers to identify patients with an increased risk of developing diabetes mellitus type 1. The combined detection of antibodies against GAD65, IA2, ZnT8 and insulin is considered an important method for diagnosing diabetes mellitus type 1 at the onset of the disease.

Glutamic acid decarboxylase (GAD) catalyzes the synthesis of the neurotransmitter GABA in the brain and in the beta cells. Two isoforms of the enzyme are known: GAD65 with a molecular weight of 65 kDa and GAD67 with 67 kDa, respectively. Antibodies directed against GAD65 are observed in the majority of patients with diabetes mellitus type 1 and in a large number of individuals in the prediabetic phase. In contrast, antibodies directed against both GAD isoforms are found in patients with the very rare neuromuscular Stiff-man syndrome.

3 Test Principle

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay for the determination of specific antibodies. The strips of the microtiter plate are coated with test-specific antigens. If antibodies are present in the patient's sample, they bind to the antigens. A second biotinylated antigen binds the immobilized antibody. The assay utilizes the ability of antibodies to act bivalently with immobilized and soluble antigens. Streptavidin conjugated with the enzyme peroxidase detects the generated immune complex. A colorless substrate is converted into the colored product by the peroxidase. The signal intensity of the reaction product is proportional to the antibody activity in the sample. After stopping the signal intensity of the reaction product is measured photometrically.

4 Test Components

Component	Description
Microtiter plate **A** **MP** 1 piece	12 breakable microtiter strips (ready-to-use), 8 wells per strip, each well coated with recombinant human GAD_{65}
Calibrator **0 – 5** **CAL** 6 x 1.0 mL	Colored dilutions of human serum (ready-to-use; contains ProClin 950) The antibody activities are indicated on the quality control certificate.
Positive control **CII** **CONTROL | +** 1 x 1.0 mL	Colored dilution of human serum (ready-to-use; contains ProClin 950) The antibody activity is indicated on the quality control certificate.
Sample diluent **C** **DIL** 1 x 20 mL	Colored solution (ready-to-use; contains ProClin 950)
GAD_{65}-Biotin **H** **START** 1 x 0.2 mL	Concentrated start reagent biotinylated GAD_{65} (contains sodium azide)
Diluent for GAD_{65}-Biotin **J** **BUF H** 1 x 20 mL	Solution (ready-to-use; contains ProClin 950)
Streptavidin-per-oxidase (SA-POD) **D** **CONJ** 1 x 0.2 mL	Concentrated streptavidin conjugated to horseradish peroxidase (100x)
Diluent for SA-POD **G** **BUF D** 1 x 20 mL	Solution (ready-to-use; contains ProClin 950)
Wash buffer **B** **WASHB** 1 x 100 mL	Concentrated solution (10x; contains ProClin 950)
Substrate **E** **SUB** 1 x 15 mL	3,3´,5,5´-Tetramethylbenzidine (ready-to-use)
Stop solution **F** **STOP** 1 x 15 mL	0.25 M Sulfuric acid (ready-to-use)

Adhesive Foil 2 pieces	-
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL), multi-channel pipettes (100 – 1000 µL) and disposable pipette tips
- Graduated cylinders (100 – 1000 mL)
- Vortex mixer or other rotators
- Microtiter plate shaker
- Microtiter plate washer or wash comb
- Microtiter plate reader with optical filters for 450 nm and 620 nm or 690 nm
- Adsorbent paper or paper towel
- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps. Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the microtiter plate must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Microtiter Plate

The microtiter plate is sealed in an aluminium bag. Unused test strips should always be stored refrigerated and protected from moisture with the desiccant in the properly sealed aluminum bag. Carefully resealed, the test strips can be used for 8 weeks after opening.

8.1.2 Calibrators

The calibrators are ready-to-use and must not be diluted any further. Calibrators must be used in each test run.

8.1.3 Controls

The positive control is ready to use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.4 GAD_{65}-Biotin (H) and Diluent (J)

A sufficient amount of GAD_{65}-Biotin solution must be prepared by diluting x mL GAD_{65}-Biotin (H) with y mL diluent for GAD_{65}-Biotin (J) directly before use, For exact dilution volumes x and y see certificate of analysis supplied with the kit. The dilution ratio for manual and automated processing can be different. The GAD_{65}-Biotin solution prepared is to be used within one day and must not be stored.

8.1.5 Streptavidin-peroxidase (D) and Diluent (G)

A sufficient amount of streptavidin-peroxidase solution must be prepared by diluting SA-POD concentrate (D) 1 + 99 (e. g. 0.1 mL SA-POD concentrate with 9.9 mL diluent for SA-POD (G). The SA-POD solution prepared is stable up to 4 weeks at 2 °C to 8 °C.

8.1.6 Wash Buffer

The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e. g. 100 mL + 900 mL). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2 °C to 8 °C up to 30 days.

8.1.7 Substrate

The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.

8.1.8 Stop Solution

The stop solution is ready-to-use.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Pipetting Scheme

The following pipetting scheme is recommended:

	1	2	3	4
A	CAL 0	Sample 2		
B	CAL 1	Sample 3		
C	CAL 2	Sample 4		
D	CAL 3	Sample 5		
E	CAL 4	…		
F	CAL 5	…		
G	CII	…		
H	Sample 1	…		

9.2 Procedure

The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. The microtiter plate should be shortly shaken after addition of reagents.

Step	Description
1. Addition of dilution buffer	Add 100 µL ready-to-use dilution buffer into the wells of patient samples; leave the wells of calibrators and controls empty.

2. Addition of calibrators, controls and samples	Add 50 µL ready-to-use calibrators, controls and undiluted samples per well
3. Incubation	Cover the plate and incubate for 60 min. at RT while shaking at 500 rpm on a plate shaker
4. Preparation of reagents	Prepare sufficient volumes of reagents (B, D/G, H/J)
5. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
6. Addition of start reagent	Add 100 µL of diluted GAD_{65}-Biotin solution (prepared from H and J) to each well
7. Incubation	Cover the plate and incubate for 60 min. at RT while shaking at 500 rpm on a plate shaker
8. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
9. Addition of conjugate	Add 100 µL of diluted SA-POD (prepared from D and G) to each well
10. Incubation	Cover the plate and incubate for 20 min. at RT while shaking at 500 rpm on a plate shaker
11. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
12. Addition of substrate	Add 100 µL ready-to-use substrate to each well and shake the plate shortly
13. Incubation	Cover the plate and incubate for 20 min. in the dark at RT without shaking
14. Addition of Stop Solution	Add 100 µL ready-to-use stop solution to each well and shake the plate shortly
15. Analysis	Read optical density (OD) at 450 nm versus 620 or 690 nm within 30 min. after stopping the reaction

9.3 Automation

Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using the international WHO reference preparation NIBSC code 97/550. Quantitative results are expressed in IU/mL.

10.2 Quantitative Evaluation

For generation of a standard curve, the optical signals (optical density, OD) of the calibrators are plotted against their antibody activities and correlated by a 4-parameter logistic (4 PL) fit. Antibody activities of unknown samples can be derived directly from their optical signals by use of the generated standard curve.

10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- OD CAL 0 < CAL 1 < CAL 2 < CAL 3 < CAL 4 < CAL 5
- OD CAL 5 > 1.2
- The positive control must be evaluated positive and present an antibody activity within the validity range indicated on the quality control certificate.

If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

The reference ranges are indicated below:

	Interpretation
Antibody activity < 5 IU/mL	negative
Antibody activity ≥ 5 IU/mL	positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

In the rare neurological disorder, Stiff-man Syndrome (SMS) round 60 % of patients have GAD65 Abs in their serum. GAD_{65} Abs from patients with SMS have much higher titers compared with those of patients with type 1 diabetes. For this reason sera from patients with suspicion of SMS should be prediluted 1:50 or 1:100 with GAD_{65} Abs negative sera. GAD_{65} Abs occur also in cerebrospinal fluid of patients with SMS

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Analytical Sensitivity and Specificity

The Limit of Blank (LoB) was determined by multiple analysis of sample diluent. The Limit of Quantitation (LoQ) was correlated to an interassay coefficient of variation of 20 %.

	Analytical Sensitivity
Limit of Blank (LoB)	< 0.5 IU/mL
Limit of Quantitation (LoQ)	< 1.0 IU/mL

11.1.2 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities.

	Intraassay Precision		Interassay Precision	
	IU/mL	CV (%)	IU/mL	CV (%)

Sample 1	17	9,0	15	9.1
Sample 2	80	6.1	76	5.5
Sample 3	183	5.7	186	5.3

11.1.3 Measurement Range

Reliable accuracy, trueness, precision, linearity and recovery of test results have been observed within the measurement range of the assay from the LoQ to the upper calibrator in comprehensive studies. Samples with test results above the upper calibrator should be reported as >max. Samples with test results below the LoQ should be reported as <min. If test results above the upper calibrator are observed, the samples may be tested at a higher dilution. The resulting antibody activity must be multiplied with the additional dilution factor.

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

Sensitivity and specificity were assessed by the analysis of serum samples from 74 patients with diabetes mellitus type I and 57 samples from unselected blood donors.

	Diagnostic Performance
Sensitivity	90.5 %
Specificity	94.7 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 1.0 %) as a preservative, may cause skin sensitization (H317) and must not be swallowed or allowed to come into contact with skin or mucosa (P280, P333+P313).

Some of the reagents contain sodium azide (< 0.1 %) as a preservative and must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

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- Seissler J, Hatziagelaki E, Scherbaum WA: Modern concepts for the prediction of type 1 diabetes. Exp. Clin. Endocrinol. Diabetes 2001, 109, Suppl 2, 304 – 16.
- Pozzilli P, Manfrini S, Monetini L: Biochemical markers of type 1 diabetes, clinical use. Scand. J. Clin. Lab. Invest. Suppl. 2001; 235, 38 – 44.
- Schernthaner G, Hink S, Kopp HP et al.: Progress in the characterization of slowly progressive autoimmune diabetes in adult patients (LADA or type 1.5 diabetes). Exp. Clin. Endocrinol. Diabetes. 2001, 109, Suppl 2, 94 – 108.
- Winter WE, Harris N, Schatz D: Immunological markers in the diagnosis and prediction of autoimmune Type 1a diabetes. Clinical Diabetes 2002, 20, 183 – 91.

15 Symbols

	Manufacturer
	CE marking of conformity
	*In vitro* diagnostic medical device
	Catalogue number
	Unique device identifier
	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Warning
	Biological risk
	Keep away from sunlight
MP	Microtiter plate
CAL	Calibrators
CONTROL +	Positive control
ENH	Enhancer
START	Start reagent
BUF H	Diluent for start reagent
CONJ	Conjugate
BUF D	Diluent for conjugate
WASHB	Wash buffer
SUB	Substrate
STOP	Stop solution

16 Changes

Changes in current Instructions for Use	
Current Version	009/03.2023
Summary of Changes	Editorial changes in chapter 2.