INSTRUCTION MANUAL

(April 01, 2014)

Medizym® anti-ZnT8

- 96 determinations -

3791

Enzyme immunoassay for the determination
of **autoantibodies** to
Zinc Transporter 8 (ZnT8 Abs)
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 start buffer
STOP	Stop solution	BUF H	Diluent for conjugate
START	Start buffer	CAL	Calibrators
CONTROL	Control serum		

Manufactured under license to patents
including EP 1563071

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 environmental agents. 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 in all ages.

During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies, such as anti-insulin (IAA), anti-glutamic acid decarboxylase (GAD), anti-tyrosine phosphatase ICA 512 (IA_2) and zinc transporter 8 (ZnT8), are present years before the onset of type 1 diabetes and prior to clinical symptoms.

ZnT8 autoantibodies are directed principally to the C terminal domain of ZnT8 (residues 268 – 369). Human population gene polymorphism at the codon for the 325th amino acid results in the expression of three protein variants: Arginine (R) 325, Tryptophan (W) 325 and very rarely Glutamine (Q) 325. ZnT8 autoantibodies may be specific to the R 325 or W 325 variant, or may be residue 325 non-specific. Sera that react with the Q allele only are extremely rare.

The Medizym® anti-ZnT8 ELISA is capable of detecting, and quantifying, autoantibodies specific to R 325 or to W 325, or to residue 325 non-specific variants.

LITERATURE

- Wenzlau, J. M. et al.: The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes.PNAS 2007; 104: 17040-17045.
- Achenbach, P. et al.: Autoantibodies to zinc transporter 8 and Slc30A8 genotype stratify type 1 diabetes risk. Diabetologica 2009; 52: 1881-1888.

PRINCIPLE of the TEST

Medizym® anti-ZnT8 is an enzyme immunoassay for the quantitative determination of autoantibodies to zinc transporter 8 (ZnT8 Ab) in human serum.

The assay utilizes the ability of ZnT8 Abs to act divalently, and to form a bridge between immobilized ZnT8 and ZnT8-Biotin in the fluid phase.

In the first step, ZnT8 Ab present in samples binds with ZnT8 immobilized onto the microtiter plate. In the second step, ZnT8-Biotin binds to this complex. The amount of ZnT8-Biotin bound correlates with the level of antibodies present in patient samples. Unbound ZnT8-Biotin is then removed by washing. Bound ZnT8-Biotin can then be quantified by addition of streptavidin peroxidase (SA-POD) and a colorigenic substrate (TMB), and reading the optical density at 450nm.

SAMPLE PREPATION

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. The use of lipemic or hemolyzed serum samples should be avoided. Citrate and heparin plasma can also be used.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires storage at- 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, initially aliquot samples and store at - 20 °C.

TEST COMPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate** 12 breakable strips of 8 wells coated with ZnT8		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 ml		**0.7 ml** concentrate
E SUB	**Substrate (TMB)** (3,3´,5,5´-Tetramethylbenzidine)		**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	**ZnT8-Biotin**		**3 vials** lyophilized
J BUF H	**Diluent for ZnT8-Biotin (H)**		**2x 15 ml** ready for use colored red
C I CONTROL	**Negative control** For concentration: see leaflet	⚠	**0.7 ml** ready for use
C II, C III CONTROL	**Positive controls** For concentrations: see leaflet	⚠	**2 vials** **0.7 ml** each ready for use
1 - 4 CAL	**Calibrators** For concentrations: see leaflet	⚠	**4 vials** **0.7 ml** each ready for use

Materials required in addition

- 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-ZnT8 has been designed for 96 determinations. This is sufficient for the analysis of 41 unknown samples as well as for calibrators and control sera assayed in duplicate.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label. The maximum shelf life is still limited to 6 months in the moment.

Upon receipt, all components of the Medizym® anti-ZnT8 have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow samples to reach room temperature prior to assay. Allow all reagents to reach room temperature prior to assay, except ZnT8-Biotin (D) and ZnT8-Biotin reconstitution buffer (F). Take care to agitate serum samples gently in order to ensure homogeneity.

Please perform the following steps with care:

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 for max. 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 (eg. 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 the substrate solution (E) to light.

H Prepare a sufficient amount of ZnT8-Biotin solution by reconstitution of one vial lyophilized ZnT8-Biotin (H) with 5.5 ml cold (2-8°) diluent for ZnT8-Biotin (J) directly prior to use. The ZnT8-Biotin solution can be stored at 2 - 8 °C for 3 days.

ASSAY PROCEDURE

- Duplicates are recommended.

1. Pipette into the corresponding wells according to assay scheme
   - **25 µl** negative control (C I) and calibrators (1 - 4)
   - **25 µl** control sera (C II, C III) and samples.
2. Cover the plate, shake for approximately 5 seconds on a plate shaker at >500 rpm and incubate overnight, for **16 – 20 hours**, at 2 - 8°C.
3. 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.
4. Add **100 µl** of cold reconstituted ZnT8-Biotin solution (prepared from H and J) to each well.
5. Cover the plate and incubate for **60 min** at 2 - 8 °C, without shaking.
6. 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.
7. Add **100 µl** reconstituted SA-POD (prepared from D and G) to each well.
8. Cover the plate and incubate for **20 min** at room temperature (18 - 25 °C) while shaking >500 rpm.
9. 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.
10. Add **100 µl** substrate solution (E) to each well.
11. Incubate for **20 min** in the **dark** at room temperature, without shaking.
12. Add **100 µl** stop solution (F) after exactly **20 min** to each well. Shake the plates for 5 seconds at >200 rpm.
13. Read the optical density **at 450 nm** against **620 or 690 nm** with a micro plate reader, **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. Without shaking the ODs will be measured about 20% lower with a loss of sensitivity.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 on the y-axis, against their respective ZnT8-Ab concentrations on the x-axis. In addition, the negative control (C I) should be included (see below).

The ZnT8 Ab concentrations of the controls and the unknown samples are read directly in U/ml from the measured OD values.

Medizym® anti-ZnT8 may also be used with computer assisted analysis software, able to produce curves with a spline smoothing fit.

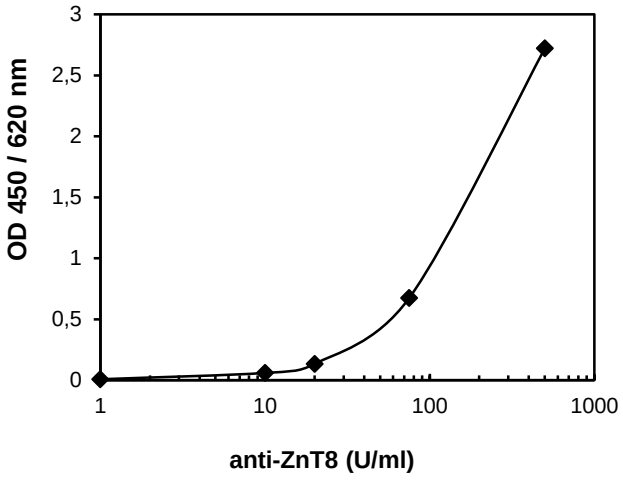
TYPICAL EXAMPLE

Do not use for evaluation!

Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	U / ml
Control **C I**	0.007	0.009	0.008	**1**
Calibrator **1**	0.058	0.063	0.060	**10**
Calibrator **2**	0.128	0.140	0.134	**20**
Calibrator **3**	0.667	0.680	0.673	**75**
Calibrator **4**	2.741	2.702	2.720	**500**
Control **C II**	0.364	0.361	0.362	50
Control **CIII**	1.580	1.612	1.596	135

STANDARD CURVE

Typical example



REFERENCE VALUES

Medizym® anti-ZnT8	
Negative	< 15.0 U/ml
Positive	≥ 15.0 U/ml

Healthy individuals should be tested negative with the Medizym® anti-ZnT8 assay. However, ZnT8 Abs may also be present in apparently healthy persons.

It is recommended that each laboratory establishes its own normal and abnormal ranges for anti-ZnT8 antibody levels, as is usually done for other parameters. Therefore, the above mentioned reference values provide only a guide.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference standard for ZnT8 antibodies, Medizym® anti-ZnT8 is calibrated in arbitrary units (U/ml).

Specificity and sensitivity

In a large study the Medizym® anti-ZnT8 kit achieved 99% (n=90) specificity and 68% (n=50) sensitivity.

Accuracy

Sera containing Rheumatoid Factor and autoantibodies to thyroglobulin, thyroid peroxidase, aquaporin-4 and the acetylcholine receptor were negative for ZnT8 Ab. One of 24 (4%) of sera positive for antibodies to the TSH receptor, and 2 of 23 (9%) of sera positive for 21-hydroxylase Ab were positive for ZnT8 antibodies using the Medizym® anti-ZnT8 kit.

No interference was observed when samples were spiked with the following materials; haemoglobin up to 500 mg/dl, bilirubin up to 20 mg/dl or intralipid up to 3000 mg/dl.

Lower Detection Limit

The negative control C I was assayed 20 times and the mean and the standard deviation were calculated. The lower detection limit at +2 standard deviations was 1.2 U/ml.

Intra and inter assay variation

Intra assay			Inter assay		
Sample no.	mean (U/ml)	CV (%)	Sample no.	mean (U/ml)	CV (%)
1	24.5	3.5	A	26.6	8.7
2	63.0	6.2	B	64.0	7.5
3	160.0	6.2	C	102.0	9.3

Linearity

Anti-ZnT8 positive human serum samples diluted with ZnT8 Ab-free human serum, measured with the Medizym® anti-ZnT8 assay, show the theoretically expected values.

On the basis of the heterogeneous nature of the autoantibody population, and with regard to epitope specificity and affinity of the autoantibodies, the theoretically expected values when diluting with ZnT8 Ab-free human serum do not always correspond with the measured concentrations.

Medizym® anti-ZnT8

ASSAY SCHEME

Bring all reagents to room temperature, except ZnT8-Biotin (H) and ZnT8-Biotin reconstitution buffer (J). Gently mix all reagents to ensure homogeneity.

Step	Activity	Material	CI / CAL 1 - 4	C II, C III	Samples 1, 2 etc.
1	Pipette	Calibrators Controls Samples	25 µl	25 µl	25 µl
2	Cover and incubate	Plate	**16 – 20 hours at 2 – 8 °C without shaking**		
3	Aspirate or decant		Tap sharply onto absorbent tissue		
	Pipette	Wash solution (produced from B)	3 x 300 µl	3 x 300 µl	3 x 300 µl
4	Pipette	**Cold** ZnT8-Biotin solution (produced from H and J)	100 µl	100 µl	100 µl
5	Cover and incubate	Plate	**1 hour at 2 – 8 °C without shaking**		
6	Aspirate or decant		Tap sharply onto absorbent tissue		
	Pipette	Wash solution (produced from B)	3 x 300 µl	3 x 300 µl	3 x 300 µl
7	Pipette	SA-POD solution (produced from D and G)	100 µl	100 µl	100 µl
8	Cover and incubate	Plate	**20 Minutes** at room temperature (18 - 25 °C) **with shaking (> 500 rpm)**		
9	Aspirate or decant		Tap sharply onto absorbent tissue		
	Pipette	Wash solution (produced from B)	3 x 300 µl	3 x 300 µl	3 x 300 µl
10	Pipette	Substrate (E)	100 µl	100 µl	100 µl
11	Cover and incubate	Plate	**20 Minutes** at room temperature **in the dark**		
12	Pipette and shake briefly	Stop solution (F)	100 µl	100 µl	100 µl
13	Measure OD		At 450 nm versus 620 (or 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.