Anti-ZnT8 (Zinc Transporter 8) ELISA

Catalog Number:
ZT831-K01
1 x 96 well ELISA kit
For Research Use Only
v. 1.0

EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

Intended Use:

The Eagle Biosciences Anti-ZNT8 (Zinc Transporter 8) ELISA Assay kit is intended for the quantitative determination of Anti-ZNT8 (Zinc Transporter 8) in serum or plasma by enzyme linked immunoassay (ELISA). The Eagle Biosciences Anti-ZNT8 ELISA Assay kit is for research use only and not to be used in diagnostic procedures.

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 325^{th} 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 Eagle Biosciences anti-ZnT8 ELISA Assay kit is capable of detecting, and quantifying, autoantibodies specific to R 325 or to W 325, or to residue 325 non-specific variants.

Principle of the Test:

The Anti-ZnT8 ELISA is an enzyme immunoassay for the quantitative determination of autoantibodies to zinc transporter 8 (ZnT8 Ab) in human serum or plasma. 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.

Samples:

Specimen collection and storage

- Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Do not use lipaemic or grossly hemolytic serum samples. EDTA 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.

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:

- Anti-ZNT8 (Zinc Transporter 8) ELISA Assay kit has been designed for 96 determinations. This is sufficient for the analysis of 41 unknown samples as well as for calibrators and controls assayed in duplicates.

- The expiry date of each component is reported on its respective label, that of the complete Anti-ZNT8 (Zinc Transporter 8) ELISA Assay kit on the box label. The maximum shelf life is still limited to 6 months in the moment.

- Upon receipt, all components of the Anti-ZNT8 (Zinc Transporter 8) ELISA Assay kit have to be kept at 2 - 8 °C, preferably in the original ANTI-ZNT8 ELISA Assay 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 patient 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. Anti-ZnT8 may also be used with computer assisted analysis software, able to produce curves with a spline smoothing fit.

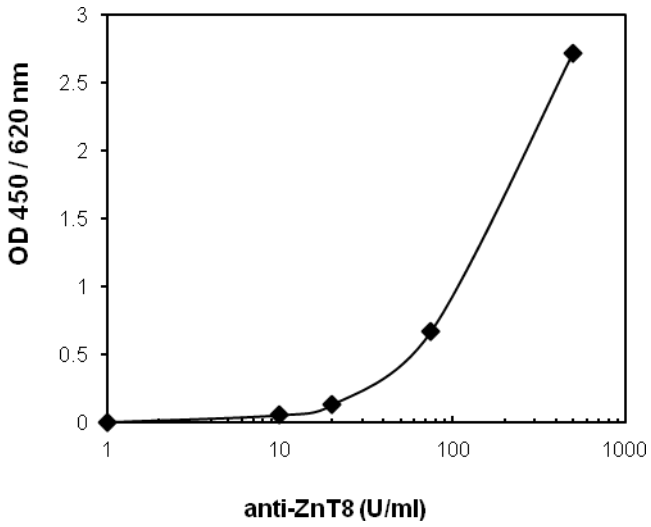
Typical Example:

Do not use for evaluation!

Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	pmol/l
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:

Anti-ZnT8	
Negative	< 15.0 U/ml
Positive	≥ 15.0 U/ml

Healthy individuals should be tested negative with the Eagle Biosciences anti-ZnT8 ELISA Assay kit. However, ZnT8 Abs may also be present in apparently healthy persons.

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

Characteristic Assay Data:

Calibration

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

Linearity

Anti-ZnT8 positive human serum samples diluted with ZnT8 Ab-free human serum, measured with the Anti-ZnT8 ELISA 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.

Assay Procedure:

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	Patient 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:

- **The Eagle Biosciences Anti-ZnT8 ELISA Assay kit is for Research 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 of the Anti ZnT8 ELISA Assay 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.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about the ANTI-ZNT8 ELISA Assay kit, its application or the procedures in this ANTI-ZNT8 ELISA Assay kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.