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User's Manual

Zinc Transporter 8 (ZnT8) Autoantibody ELISA Kit

REF **DEIABL438**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme Immuno Assay for the Quantitative Determination of ZnT8 Autoantibodies in Serum

General Description

The ZnT8 autoantibody (ZnT8 Ab) ELISA kit is intended for use for the quantitative determination of ZnT8 autoantibodies in human serum. Autoantibodies to pancreatic beta cell antigens are important serological markers of type 1 diabetes mellitus (type 1 DM). The antigens recognised by these antibodies include insulin, glutamic acid decarboxylase (GAD65 kDa isoform), the islet cell antigen IA-2 or ICA-512 and zinc transporter 8 (ZnT8). 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. ZnT8 autoantibody ELISA is capable of detecting, and quantifying, autoantibodies specific to R 325 or to W 325, or to residue 325 non-specific variants.

In ZnT8 Ab ELISA, ZnT8 autoantibodies in test patients' sera, calibrators and controls are allowed to interact with ZnT8 coated onto ELISA plate wells. After a 16 - 20 hour incubation, the samples are discarded leaving ZnT8 autoantibodies bound to the ZnT8 coated wells. ZnT8 Biotin is added in a 2nd incubation step where, through the ability of ZnT8 autoantibodies in the samples to act bivalently (or polyvalently), a bridge is formed between ZnT8 bound to the wells and ZnT8 Biotin. Unbound ZnT8 Biotin is then removed in a wash step and the amount of bound ZnT8 Biotin determined (in a 3rd incubation step) by addition of Streptavidin Peroxidase (SA-POD), which binds specifically to Biotin. Excess, unbound SA-POD is then washed away and addition of 3,3',5,5' – tetramethylbenzidine (TMB) results in formation of a blue colour. This reaction is stopped by addition of stop solution causing the well contents to turn yellow. The absorbance of the yellow reaction mixture at 405 nm and 450 nm is then read using an ELISA plate reader. A higher absorbance indicates the presence of ZnT8 autoantibody in the test sample. Reading at 405 nm allows quantitation of high absorbances. Low values (less than 50 units per ml) should be read off the 450 nm calibration curve. The measuring range is 10 – 2000 u/ml.

Reagents And Materials Provided

1. ZnT8 Coated Wells: 8*12 strips
2. Calibrators A – E: 0.7 ml each, ready for use
3. Positive Controls: .7 ml, ready for use, 2 vials
4. Negative Control: 0.7 ml, ready for use
5. ZnT8-Biotin: 5.5 ml per vial, lyoph.
6. Reconstitution Buffer: 15 ml, coloured red, ready for use
7. Streptavidin-peroxidase (SA-POD): 0.7 ml, 20 x concentrated
8. Diluent: 15 ml, ready for use
9. Substrate: 15 ml, tetramethyl benzidine (TMB), ready for use

10. Wash Buffer: 125 ml, 10 x concentrated
11. Stop Solution: 12 ml, ready for use

Materials Required But Not Supplied

- Pipettes for 25 µl and 100 µl
- ELISA plate cover
- ELISA plate shaker capable of 500 shakes per min (not an orbital shaker)
- Pure water
- Microtiter plate reader (450 nm and 405 nm)

Storage

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Evaluation

In the IASP 2015 study the ZnT8 Ab ELISA kit achieved 97% (n=90) specificity and 76% (n=50) sensitivity.

Assay of 297 healthy blood donor sera gave a mean value of 1.9 ± 3.84 U/ml. 3 sera (1%) were above the assay cut off giving values of 45, 41 and 19 U/ml.