DIAGNOSTIKA GMBH

Instructions for Use

Titin Antibody ELISA

Enzyme Immunoassay for the
Semi-Quantitative Determination of
Anti-Titin-Antibodies in Serum and Plasma

CE IVD

Item No. EA601/48

 6 x 8

 2 – 8 °C

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Table of Contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of Conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue Number of Manufacturer		Consult Instructions for Use

Hazard Pictograms

Warning

1 Introduction and Principle of the Test

In about 80 % of patients with Myasthenia gravis alterations of the thymus can be detected. Approximately 10 % of these patients develop thymus neoplasia as thymic epithelial tumor (TET) or thymic carcinoma. An early diagnosis of the thymoma and subsequent thymectomy is crucial for the prognosis of these patients.

Most of these patients develop, in addition to the acetylcholine receptor antibodies detected in myasthenia gravis (ACHRAB®-Assay), other autoantibodies against striated muscle, including antibodies against titin.

Titin is a protein of the striated muscles with an extremely high molecular weight. The immunogenic region of titin is located on a 30 kD protein fragment. Antibodies against this fragment presumably crossreact with the epitopes of the acetylcholine receptors (paraneoplastic myasthenia gravis). The recombinant MGT30 peptide is used in the ELISA for the specific determination of anti-titin antibodies.

This ELISA test is superior to the immunofluorescence test (IFT) on sections of striated muscle (human, monkey).

The Titin Antibody ELISA is a sandwich enzyme immunoassay. During sample incubation, anti-titin antibodies in the diluted patient samples and standards bind to recombinant titin fragments (MGT30 peptide) immobilized on the surface of the microtiter plate wells.

After a wash step, peroxidase-labeled protein A is added, which binds to the anti-titin antibodies. After another wash step, the amount of enzyme bound is determined by adding tetramethylbenzidine (TMB), which is converted into a blue dye. The addition of sulfuric acid stops the reaction, thereby causing a color change to yellow.

The absorbance of each sample is then measured with a microplate photometer at 450 nm and evaluated using the calibrator provided with the test.

2 Precautions

- For in vitro diagnostic use only. For professional use only.
- Before carrying out the test, the instructions for use, as included in the kit, should be read completely and the content understood.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy; however, these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and patient samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and eye protection while performing the test.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- The quality control guidelines in the medical laboratory regarding the inclusion of control samples and/or pooled samples should be observed.

3 Storage and Stability

The kit is shipped at ambient temperature and is subsequently stable until the stated expiry date when stored between 2 - 8 °C. Once opened, the kit is stable until the expiry date.

The shelf life of the ready-to-use reagents is indicated on the respective bottle label. The shelf life and storage conditions of the prepared reagents is stated under 6.1.

Bring all reagents to room temperature before use and refrigerate immediately after use.

4 Contents of the kit

MT-Strips **STRIPS** 6 strips
8 wells each, break apart
Precoated with recombinant MGT30 peptide

Calibrator **CAL** 1 vial
1 ml prediluted serum (1:101), ready for use

Negative Control **CON -** 1 vial
1 ml prediluted serum (1:101), ready for use

Positive Control **CON +** 1 vial
1 ml prediluted serum (1:101), ready for use

Sample Diluent **DIL** 1 bottle
55 ml, ready for use

Enzyme Conjugate **CONJ** 1 vial
6 ml, ready for use, Protein-A-POD conjugate  Warning

Wash Buffer **WASH** 1 bottle
50 ml, concentrate (10x)

Substrate **SUB** 1 vial
6 ml TMB solution, ready for use

Stop Solution **STOP** 1 vial
6 ml, ready for use
Contains 0.3 M sulphuric acid

Materials required but not provided:

- Pipettes (10 µl, 100 µl, 1 ml)
- Repeating dispenser
- Horizontal shaker
- Multichannel pipette or microtiter plate washing device
- Microplate photometer (450 nm and 405 nm)

- Distilled water
- Vortex mixer, roller mixer
- Paper towels, pipette tips, timer

5 Sample Collection and Storage

5.1 Serum and Plasma

Only serum or plasma should be used for the test. Follow the instructions provided by the manufacturer of the blood collection system. The usual precautions for blood collection must be observed.

Freshly collected samples should be used. If necessary, the serum or plasma samples can be stored at -20 °C. Avoid repeated freezing and thawing. Hemolytic, lipemic and iteric samples should not be used.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

Allow all kit contents to reach room temperature.

6.1.1 Wash Buffer

Dilute the Wash Buffer WASH (50 ml) with distilled water to a final volume of 500 ml, mix briefly.

The diluted wash buffer can be stored at 2 - 8 °C for a maximum period of 4 weeks.

If the kit is to be divided into several runs, prepare for each run only the required amount of wash buffer.

All other reagents are ready for use.

6.2 Preparation of Samples

Samples must be diluted 1:101 in Sample Diluent DIL (e.g. 10 µl serum plus 1 ml sample diluent) before assaying. In case diluted samples shall be re-assayed, freeze the diluted samples.

7 Assay Procedure

1. Pipette each 100 µl of ready for use Calibrator CAL, ready for use Negative Control CON - and Positive Control CON + and 1:101-diluted samples (s. 6.2) into the corresponding wells STRIPS. Duplicates are recommended. Place unused microtiter strips back into the foil pouch together with the desiccant and seal the pouch carefully.
2. Incubate for 60 minutes at room temperature (20 - 25 °C) on a horizontal shaker at medium shaking frequency.
3. Discard or aspirate the contents of the wells and wash with 300 µl diluted Wash Buffer WASH per well. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times. Alternatively, a microtiter plate washing device can be used.
4. Pipette 100 µl Enzyme Conjugate CONJ into each well.
5. Incubate for 30 minutes at room temperature (20 - 25 °C) on a horizontal shaker at medium shaking frequency.
6. Repeat the washing procedure as described in 3.
7. Pipette 100 µl Substrate SUB into each well.
8. Incubate for 20 ± 5 minutes at room temperature (20 - 25 °C) on a horizontal shaker at medium shaking frequency.
9. Add 100 µl Stop Solution STOP to each well. Take care to pipette in the same order and speed as in step 7. Shake for 10 seconds on a horizontal shaker.
10. Within 15 minutes, read the optical density at 450 nm (reference wavelength between 570 nm and 650 nm) in a microplate photometer.
    Samples with an OD at 450 nm higher than 2.5 should be read again at 405 nm (reference wavelength between 570 nm and 650 nm) and should be evaluated against the calibrator read at 405 nm.

8 Calculation of Results

The measured optical density (OD) of the samples divided by the optical density of the Calibrator equals factor F:

$$\text{Factor}_{\text{sample}} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{calibrator}}}$$

Samples with an OD at 450nm higher than 2.5 should be read again at 405nm and should be related to the calibrator OD read at 405nm.

Patient values (factor F) ≥ 1.0 are considered positive and all values < 1.0 are considered negative.

Example (do not use for calculation of results):

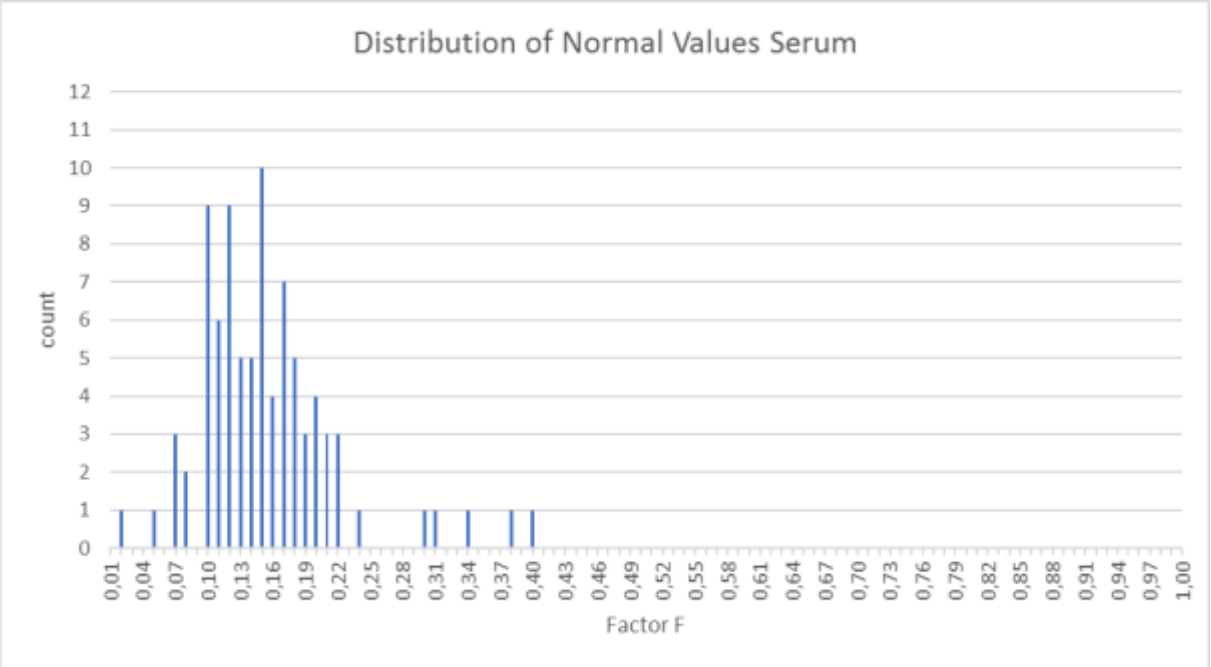
	$\mathbf{OD_{sample}}$	$\frac{\mathbf{OD_{sample}}}{\mathbf{OD_{calibrator}}}$	**Interpretation**
Calibrator	1.241	1.0	
Negative Control	0.287	0.23	**–**
Positive Control	2.346	1.89	**+**
Sample 1	0.826	0.67	**–**
Sample 2	1.710	1.38	**+**

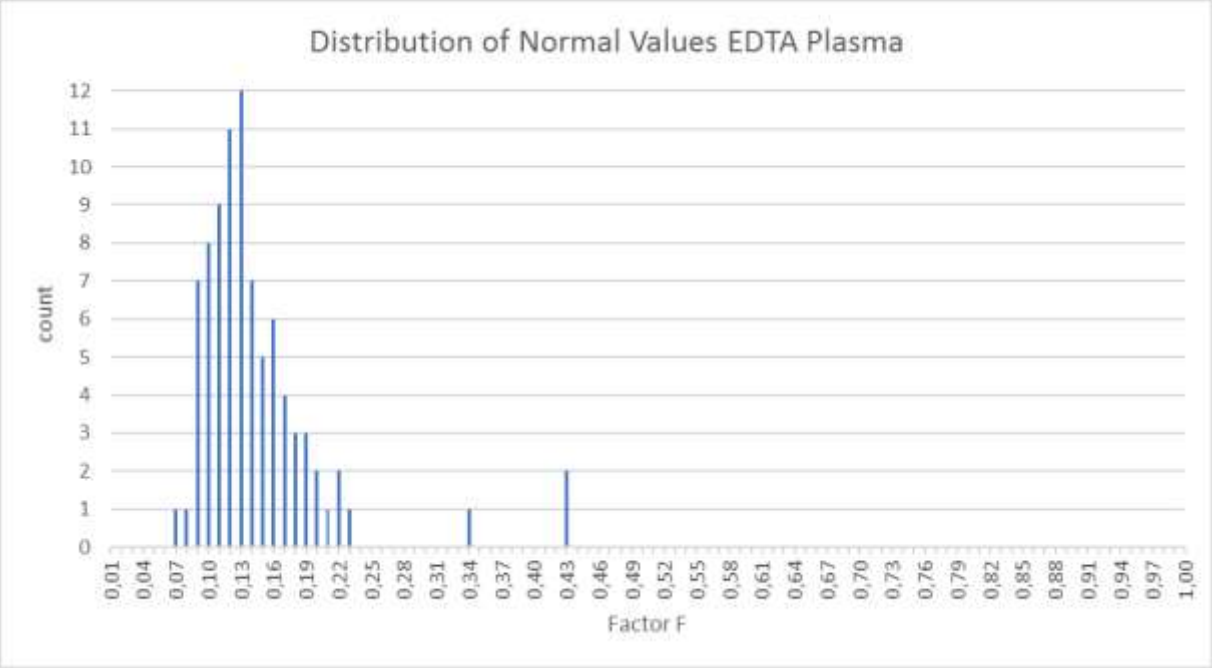
9 Assay Characteristics

In the following the results of determining each 86 serum and EDTA plasma samples of normal healthy blood donors are shown. The following graphs show the distribution of the normal values.

The reference range is given as factor F < 1.0.

Distribution of normal values





9.1 Reproducibility

Reproducibility was determined by calculating the intra- and inter-assay coefficients of variation.

Values are given as $OD_{sample}/OD_{calibrator}$.

9.1.1 Intra Assay

sample	n	mean	sd	cv (%)
1	40	1.87	0.054	2.9
2	40	0.19	0.005	2.4

9.1.2 Inter Assay

sample	n	mean	sd	cv (%)
1	20	0.11	0.015	14.0
2	20	0.11	0.010	9.2
3	20	0.20	0.025	12.7
4	20	0.69	0.055	8.1
5	20	1.03	0.102	9.9
6	20	1.03	0.089	8.6
7	20	1.06	0.102	9.6
8	20	1.09	0.082	7.5
9	20	1.47	0.089	6.1
10	20	1.46	0.093	6.4
11	20	1.68	0.099	5.9
12	20	2.00	0.197	9.8

9.2 Linearity

Samples containing titin antibodies do not dilute in a linear manner in the Titin Antibody ELISA.

9.3 Limitations of the method

The results of the Titin Antibody ELISA are to be evaluated together with further diagnostic procedures and the medical history and the question posed.

9.4 Interferences

Hemolytic, lipemic and icteric samples should not be used.

10 Literature

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  Neurology 1997;49:1454-1457

- M Gautel, A Lakey, DP Barlow, Z Holmes, S Scales, K Leonard, S Labeit, A Mygland, NE Gilhus, JA Aarli
  Titin antibodies in myasthenia gravis: Identification of a major immunogenic region of titin
  Neurology 1993;43:1581-1585

11 Changes to declare

Version _10: Additions and changes in phrasing have been made to provide greater clarity and are highlighted in gray.

Version _9: The manufacturer and distributor information have been changed. No changes have been made to components or execution of protocols.

Version _8: IFU has been re-formatted. Parts of the sections and pipetting schemes have been rephrased to provide greater clarity. No changes have been made to components or execution of protocols.

Pipetting Scheme

		Calibrator	Controls	Samples
STRIPS:				
CAL	µl	100		
CON - & CON +	µl		100	
Diluted Samples	µl			100

60 minutes incubation at room temperature, with shaking
4 x washing

CONJ	µl	100	100	100

30 minutes incubation at room temperature, with shaking
4 x washing

SUB	µl	100	100	100

20 ± 5 minutes incubation at room temperature, with shaking

STOP	µl	100	100	100

Shake for 10 seconds
Read absorbance at 450 nm (ref. 570 – 650 nm)