



## Instructions for Use

# Titin Antibody ELISA

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




Item No. EA601/48



6 x 8



2 – 8 °C

**REF** TIT00  Wesamin GmbH & Co. KG • Graff 1 • 24568 Oersdorf • Germany

Distributor: DLD Diagnostika GmbH • Adlerhorst 15 • 22459 Hamburg • Germany

Tel. +49 40 5558710 • Fax +49 40 55587111 • [contact@dld-diagnostika.de](mailto:contact@dld-diagnostika.de) • [www.dld-diagnostika.de](http://www.dld-diagnostika.de)

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## Symbols



In Vitro Diagnostic  
Medical Device



EC Declaration of Conformity



Content



Expiry Date



Lot Number



Store at



Manufactured by



Sufficient for ... determinations



Catalogue Number of  
Manufacturer



Consult Instructions for Use

The symbols of the components of the kit are described in chapter 4 Contents of the Kit.

## 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

<b>MT-Strips</b> 8 wells each, break apart Precoated with recombinant MGT30 peptide	<b>STRIPS</b>		6 strips
<b>Calibrator</b> 1 ml prediluted serum (1:101) ready for use	<b>CAL</b>		1 vial Warning
<b>Negative Control</b> 1 ml prediluted serum (1:101) ready for use	<b>CON -</b>		1 vial Warning
<b>Positive Control</b> 1 ml prediluted serum (1:101) ready for use	<b>CON +</b>		1 vial Warning
<b>Sample Diluent</b> 55 ml, ready for use	<b>DIL</b>		1 bottle
<b>Enzyme Conjugate</b> 6 ml, ready for use, Protein-A-conjugated peroxidase	<b>CONJ</b>		1 vial Warning
<b>Wash Buffer</b> 50 ml, concentrate (10x)	<b>WASH</b>		1 bottle
<b>Substrate</b> 6 ml TMB solution, ready for use	<b>SUB</b>		1 vial
<b>Stop Solution</b> 6 ml, ready for use Contains 0.3 M sulphuric acid	<b>STOP</b>		1 vial

Additionally included: Adhesive Foil

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 EDTA-Plasma

Only serum or EDTA-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 icteric 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 (see 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. Seal the plate with adhesive foil and 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] (see 6.1.1) 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 at least 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)  $\geq 1.0$  are considered positive and all values  $< 1.0$  are considered negative.

Example (do not use for calculation of results):

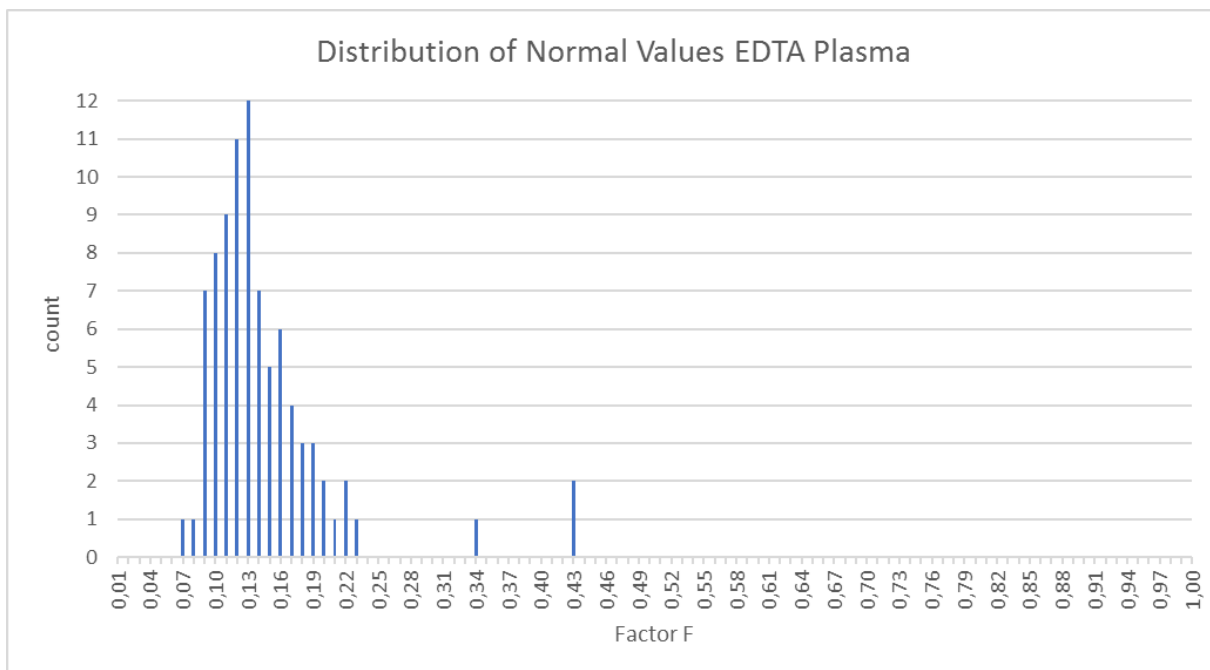
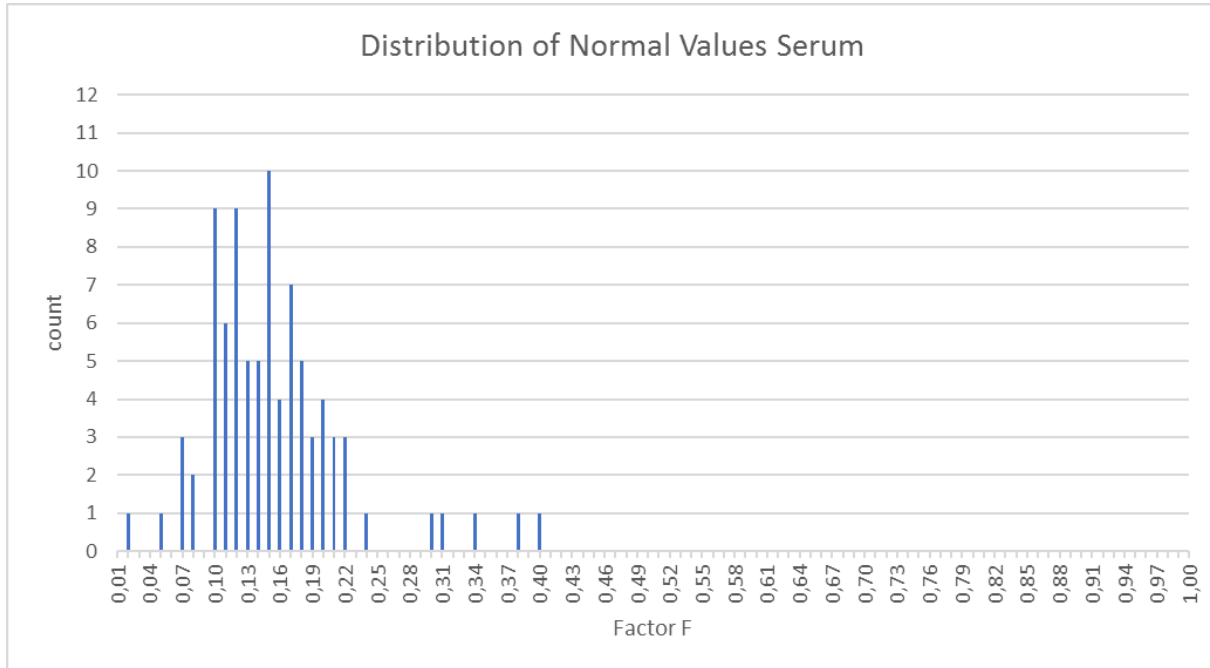
	<b>OD<sub>sample</sub></b>	<b><math>\frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{calibrator}}}</math></b>	<b>Interpretation</b>
Calibrator	1.241	1.0	
Negative Control	0.287	0.23	-
Positive Control	2.346	1.89	+
Sample 1	0.826	0.67	-
Sample 2	1.710	1.38	+

## 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  $OD_{\text{sample}}/OD_{\text{calibrator}}$ .

### 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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**Striational autoantibodies in myasthenia gravis patients recognize I-band titin epitopes**  
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- RD Voltz, WC Albrich, A Nägele, F Schumm, M Wick, A Freiburg, M Gautel, HT Thaler, J Aarli, Th Kirchner, R Hohlfeld  
**Paraneoplastic myasthenia gravis: Detection of anti-MGT30 (titin) antibodies predicts thymic epithelial tumor**  
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 \_12: Changes are highlighted in gray.

Version \_11: Changes are highlighted in gray.

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

Seal plate with adhesive foil

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

CONJ	μl	100	100	100
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30 minutes incubation at room temperature, with shaking  
4 x washing

SUB	μl	100	100	100
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20 ± 5 minutes incubation at room temperature, with shaking

STOP	μl	100	100	100
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Shake for minimum 10 seconds  
Read absorbance at 450 nm (ref. 570 – 650 nm)